A Laboratory Manual of Physiological Chemistry

WEBSTER & KOCH

QP
519
W38

Cornell University Library

BOUGHT WITH THE INCOME
FROM THE
SAGE ENDOWMENT FUND
THE GIFT OF
Henry W. Sage
1891

A. 234177 21/V/09

3513-1

The date shows when this volume was taken.
To renew this book copy the call No. and give to the librarian.

HOME USE RULES.

All Books subject to Recall.

Books not used for instruction or research are returnable within 4 weeks.

Volumes of periodicals and of pamphlets are held in the library as much as possible. For special purposes they are given out for a limited time.

Borrowers should not use their library privileges for the benefit of other persons.

Books not needed during recess periods should be returned to the library, or arrangements made for their return during borrower's absence, if wanted.

Books needed by more than one person are held on the reserve list.

Books of special value and gift books, when the giver wishes it, are not allowed to circulate.

Readers are asked to report all cases of books marked or mutilated.

Do not deface books by marks and writing.

Cornell University Library
QP 519.W38

Laboratory manual of physiological chemi

3 1924 024 545 208 olin

Cornell University Library

The original of this book is in
the Cornell University Library.

There are no known copyright restrictions in
the United States on the use of the text.

http://www.archive.org/details/cu31924024545208

MANUAL OF PHYSIOLOGICAL CHEMISTRY

A LABORATORY MANUAL OF PHYSIOLOGICAL CHEMISTRY

BY

RALPH W. WEBSTER, M.D., PH.D.
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE UNIVERSITY OF CHICAGO

AND

WALDEMAR KOCH, PH.D.
DEPARTMENT OF PHARMACOLOGY
THE UNIVERSITY OF CHICAGO

Chicago: The University of Chicago Press
London: William Wesley & Son
1903

D/va

21/V/09

A 224 7

COPYRIGHT, 1903
THE UNIVERSITY OF CHICAGO

INTRODUCTION.

The rapid development of the science of bio-chemistry, and the establishment of closer relationships on the one hand with the pure sciences of physics, chemistry, zoölogy, pathology, and botany, and on the other with clinical chemistry and medicine, make it necessary that the science be presented in a broader way than has hitherto been the case. Instruction in physiological chemistry is still confined largely or entirely to the subjects of the foods, digestion, the blood, and the urine of mammals, and is given exclusively to medical students. Confinement within such narrow bounds has not only been detrimental to the science itself, which is one of the broadest and most fundamental of all the biological sciences, but it has been equally injurious to the development of scientific therapeutics and to physiology, since wide fields of research which might be expected to yield discoveries of value to physiology and pathology have been neglected.

At this University an attempt has been made to bring physiological chemistry into closer touch with the biological sciences, without unduly sacrificing connection with the clinical branches; and this Manual represents the laboratory course which is given to students who wish general instruction in the subject, which shall be not too remote from its practical applications. The Manual so prepared is necessarily a compromise between ideals and their practical applications. The laboratory course is supplemented by lectures in which the general chemistry and chemical properties of animal and plant protoplasm are developed more at length, and by a course in clinical and pathological chemistry which is taken by medical students only.

It will be seen by an inspection of the index that the subject is developed using the mammal as a type of living organisms. This is purely for the sake of convenience, since the greater number of students who take the course are men preparing for medicine, who must know mammalian chemistry. It is hoped, however, in subsequent editions to develop the general chemistry of protoplasm and protoplasmic processes as illustrated by bacterial and yeast fermentations, and to bring the subject into still closer touch with dynamic chemistry. The course begins with the study of the chemistry of protoplasm, using yeast as an example. The dependence of the reactions going on in yeast and other protoplasm upon the environment, and the principles of chemical equilibrium, are then taken up. This is followed by a study of the foods or raw products; their changes during cooking, digestion, and absorption; the chemistry of the blood and the master tissues of the body; and finally the chemistry of the excretions. This development of the subject is not only logical, but has been found to work very well in practice.

More experiments and methods are given than can possibly be performed by any regular medical student. These were introduced that the book might serve as a convenient manual for those desiring to go farther into the subjects in a supplementary course, and to give some latitude of choice to the instructor. The experiments required of the students here are printed in heavy type. Certain omissions will be noticed, such, for example, as work on the cryoscopy and physical chemistry of the blood and urine. These have been omitted for the reason that in the authors' opinion these subjects have not been sufficiently developed to make laboratory experiments by large classes of students desirable or feasible, although some instruction in the cryoscopic methods to special students may be advantageous. Such additional work may be given in optional courses.

In closing I wish to thank the authors of this Manual for their courtesy in asking me to write an introduction for it.

ALBERT P. MATHEWS.

TABLE OF CONTENTS.

INTRODUCTION v

CHAPTER I. THE CHEMISTRY OF THE CELL 1

Preparation and reactions of the primary cell constituents. Application of the laws of physical chemistry to life-phenomena.

CHAPTER II. THE CHEMISTRY OF THE FOODS 12

The physical and chemical properties of the fats, carbohydrates, and proteids. The preparation and study of serum-albumin, serum-globulin, syntonin, casein, mucin, collagen, and splitting products of proteids; protalbumose, deutero-albumose, peptone, leucin, tyrosin, hexon bases, and cystin.

CHAPTER III. THE CHEMISTRY OF DIGESTION 27

Salivary, gastric, and pancreatic digestion. Study of bile and intestinal juice.

CHAPTER IV. THE CHEMISTRY OF THE TISSUES 42

Blood: Clinical examination. Preparation of oxyhæmoglobin, fibrinogen, serum-albumin, and serum-globulin (see chap. 2).

Liver: Preparation of glycogen, nucleo-proteid, and urea.

Kidney: Preparation of cystin and urea.

Bone: Preparation of collagen (see chap. 2). Tests for inorganic constituents.

Muscle: Preparation of syntonin (see chap. 2), kreatin, paramyosinogen, myoglobulin, myo-albumin, myosin, xanthin bases, and sarcolactic acid.

Brain: Preparation of lecithin, cephalin, cholesterin, and cerebrin.

Pancreas: Preparation of trypsin.

Stomach: Preparation of pepsin.

CHAPTER V. THE CHEMISTRY OF MILK. CHEMICAL AND SANITARY EXAMINATION 57

CHAPTER VI. THE CHEMISTRY OF THE EXCRETIONS 62

Urine: Composition and clinical examination of normal and abnormal urine.

Fæces: Clinical examination.

PLATES I-XXI 99

INDEX 105

CHAPTER I.

THE CHEMISTRY OF THE CELL.

The cell as the physiological unit is made up of a mixture of substances, among which the enzymes are very important, and at the same time little known chemically. The more highly differentiated groups of cells usually contain some well characterized substances, not found in other cells. Certain classes of chemical compounds, however, are found in all cells, and are therefore of more general importance in the study of life-phenomena. Among these may be mentioned: proteids, nucleic acids, carbohydrates, fats, lecithans, cholesterin, inorganic salts, and water.

We will first study the chemical constituents of protoplasm, and the physical and chemical conditions necessary for the life of a simple living substance such as the yeast cell.

EXPERIMENT I.

Lecithin.—Two yeast cakes are ground in a mortar with 100 c.c. of alcohol, transferred to a flask, and heated on water-bath for one hour. Filter hot. Extract the residue with another 100 c.c. of alcohol for one hour. Filter hot and allow the residue to stand with 50 c.c. of ether in a stoppered flask over night. Filter and save the residue for nucleic acid. The various filtrates are united and evaporated on the water-bath. Extract this latter residue with 20 c.c. of cold ether, filter, and add three times the volume of acetone. A waxy substance precipitates, which quickly darkens on exposure to air. The substance is a mixture of lecithin and cephalin.

For the various tests a larger quantity of lecithin is prepared from eggs.

Preparation of egg lecithin.—The yolks of two eggs are separated from the white. Dilute egg white with ten times its volume of water. Filter and save the solution for proteid tests (p. 4).

The yolks are allowed to stand with 60 c.c. of ether over night; in the morning 100 c.c. of alcohol are added, and the solution is filtered. The filtrate is evaporated on the water-bath. The residue from the filtrate is dissolved in 15 c.c. of ether, filtered, and 50 c.c. of acetone are added to the filtrate. Filter through a small filter and save the filtrate for cholesterin. With the lecithin thus obtained perform the following experiments. Repeat the tests with lecithin from yeast.

1. Add a small piece to water; shake; notice cloudy appearance of water. No true solution takes place, but an emulsion is formed which can be filtered unchanged. Notice myelin forms, under microscope.

2. *Test for phosphorus.*—Fuse one-third of your lecithin with a fusion mixture in a porcelain crucible. When well fused, allow to cool, dissolve in water, acidulate with nitric acid, and add ammonium molybdate. A yellow precipitate of ammonium phosphomolybdate shows presence of phosphorus.

3. *Test for fatty acid.*—Heat one-third of your lecithin with 20 c.c. of sodium alcoholate in a flask for one hour; evaporate the alcohol. Notice the residue; it is soap. Dissolve this in water, boil, and notice the formation of soap-bubbles. Make the solution acid and allow it to stand in the warm closet over night. Fatty acids collect at the surface. Remove by filtration and recrystallize from alcohol.

4. *Test for glycerin.*—Fuse a small portion of lecithin with some potassium bisulphate. Note the peculiar irritating odor of acrolein given off. This shows presence of glycerin (in absence of sugar).

5. *Test for nitrogen.*—Fuse some lecithin in a test-tube with a piece of sodium the size of a pea. Notice the smell of ammonia and test with a moist piece of red litmus paper. The cholin present in the lecithin molecule is broken up by the metallic sodium giving off ammonia or some of the methyl-amines.

Besides these reactions, lecithin will give, with an alcoholic solution of cadmium chloride, a white precipitate of the double salt. With osmic acid it gives a black precipitate of metallic

osmium, a reaction characteristic of all fats containing oleic acid. State in your notebook your conclusions as to the nature of lecithin. Consult your text-book for its formula.

EXPERIMENT II.

Cholesterin.—The acetone solution from which the lecithin has been precipitated is evaporated on the steam-bath. The residue is dissolved in 50 c.c. of alcoholic sodium hydrate (10 per cent.) and heated on the steam-bath in a flask until all the alcohol has evaporated. The residue is dissolved in 50 c.c. of water, transferred to an evaporating dish, 10 g. of sodium chloride added, and again evaporated to complete dryness. This residue is then ground fine in a mortar, dried at 105° C., and extracted with 30 c.c. of cold ether. The ether solution is filtered and should leave on evaporation crystals of cholesterin.

Reactions of cholesterin.

1. Evaporate with nitric acid. A yellow mass is obtained which turns brick-red on addition of ammonia.

2. Mixed in the dry state with strong sulphuric acid, it produces a blue-red or violet color on addition of chloroform, the color changing to green on exposure to air.

3. Evaporated with a mixture of two volumes of sulphuric acid and one volume of ferric chloride solution, it turns violet.

4. Dissolve in chloroform and observe crystals on evaporation, under microscope. Make drawing of crystals in your notebook, and consult text-book for chemical nature of cholesterin. See Plate II.

Some cholesterin may also be obtained in the same manner from the acetone solution derived from yeast.

EXPERIMENT III.

Nucleic acid.—Take the yeast residue from the alcohol-ether extraction and treat it with 50 c.c. of 10 per cent. sodium carbonate solution for one hour at 30° to 40°. Filter and allow to cool. Save the residue for inorganic salts. After the filtrate has cooled, carefully add acetic acid until solution is slightly acid. A turbidity will be noticed, due to precipitation of the nucleo-albumins present in slight amount in the yeast. Test the action of heat on a part of this solution. Clearing of the solution may be due to clumping of the precipitate, or to the presence of too much acid. The main bulk of the solution is filtered from the nucleo-albumins and poured into twice its volume of alcohol, to which has been added 2 c.c. of concentrated hydrochloric acid for each 100 c.c. of alcohol. A white flocculent precipitate of nucleic acid is soon observed. As soon as the precipitate settles, the supernatant liquid is decanted off, the precipitate is washed with alcohol by decantation, and is filtered through a small filter.

Examine the properties of nucleic acid. Test its solubility, while moist, in water (hot and cold), in alkalies, in mineral acids, in alcohol. To the hot watery solution of nucleic acid add sodium hydrate until the reaction is alkaline. Then add a few drops of copper sulphate solution and notice color. A violet color shows presence of albumin, and hence an impurity in the nucleic acid, as this latter does not give the coloration. Examine nucleic acid for phosphoric acid by usual method. With albumins nucleic acid forms compounds, nucleo-proteids, insoluble in dilute acetic acid.

EXPERIMENT IV.

Proteids in yeast.—Take one cake of yeast, mix well with 100 c.c. of ordinary tap water at room temperature, grind up with sand, and let stand for twenty-four hours in a stoppered flask. In order to prevent decomposition, add ten drops of chloroform to the water. After the extraction is complete, filter until a perfectly clear filtrate is obtained. By this treatment some of the proteid matter of the yeast has gone into solution in the water.

Perform the following proteid tests, first with dilute white of egg solution (p. 1), then repeat with the above proteids from yeast. Notice variations and explain them.

1. *Coagulation test.*—Take three test-tubes and place in each a few cubic centimeters of the above solution. To one add a few drops of dilute acetic acid and boil. To a second add a few drops of dilute sodium hydrate and boil. Boil the third as it is without addition of any other solution. What differences, if any, are noticed? Conclusions drawn from this?

2. *Behavior toward mineral acids at ordinary temperature.*—Test action of nitric acid, hydrochloric acid, and sulphuric acid on proteid solution.

3. *Action of salts of heavy metals, such as copper sulphate, lead acetate, mercuric chloride?*

4. Add to the proteid solution a small amount of potassium ferro-cyanide and then dilute acetic acid. What occurs?

5. Add to the neutral or faintly acid proteid solution a small amount of alcohol. What do you observe? Does the precipitate dissolve again on addition of water?

6. Action of alkaloidal reagents? Picric acid, potassium mercuric iodide, phospho-tungstic acid. Use in acid solution.

7. *Millon's reagent* (solution of mercury in strong nitric acid in excess).—Add to the proteid solution three or four drops of Millon's reagent. Notice the white precipitate first formed. Boil and observe the pink or blood-red color of the precipitate and the solution. The color developed on boiling is the characteristic part of this test and depends on the presence of the tyrosin group in the proteid.

8. *Xanthoproteic reaction.*—Add strong nitric acid to the proteid solution. What occurs? Boil and notice change. Allow the mixture to cool and then add ammonium hydrate. Observe decided change of color. (Tyrosin.)

9. *Adamkiewicz's reaction.*—Add to some of the proteid solution a mixture of one volume concentrated sulphuric acid and two volumes glacial acetic acid. Observe the reddish-violet coloration which appears on heating. This reaction is obtained better with the dry proteid. Use a good excess of acid. The reaction is due to the presence of glyoxylic acid and depends on the indol or tryptophan group.

10. *Biuret test.*—Add to 5 c.c. of proteid solution a few drops of sodium hydrate and then one or two drops of dilute copper sulphate solution. On heating, a reddish-violet and finally a violet color appears in the solution. This is one of the most characteristic reactions, and depends on diacid amids in the albumin molecule. Also given by protamine which consists only of hexon bases.

11. *Liebermann's reaction.*—On heating a proteid solution with a few drops of concentrated hydrochloric acid, a violet color is produced. If a precipitate is formed first, this must be re-dissolved by the addition of more acid. This test depends on the carbohydrate group present in some proteids.

EXPERIMENT V.

Carbohydrates in yeast.—The carbohydrate most commonly found in animal cells and according to recent reports, also in the yeast cell, is glycogen. On account of the practice of mixing compressed yeast with cornstarch or dextrin, the glycogen cannot conveniently be tested for. The general reactions for monosaccharides are performed as follows:

One-fourth of a yeast cake is boiled with 50 c.c. of dilute hydrochloric acid (10 per cent.) for fifteen minutes, and the solution neutralized and filtered. With the solution of glucose thus obtained make the following tests:

1. Add Fehling's solution, equal parts of *A* and *B*. Boil. Reddish cuprous oxide forms, due to the presence of glucose. Repeat the test with Haines's solution. Write formula for the reaction in your notebooks.[1]

2. In a test-tube place a little (1 g.) solid phenyl hydrazine hydrochlorate, add an equal bulk of dry sodium acetate, and into this pour 25 c.c. of the above solution. Heat on water-bath at 100° C. for one hour. Filter while hot. On cooling a yellowish needle-shaped crystalline precipitate separates, which is characteristic of glucose. What is this precipitate? Repeat with pure glucose.

3. Add potassium hydroxide solution to the glucose solution, and then a small amount of dry bismuth subnitrate. Boil. A

---

[1] Haines's solution is made up as follows:

Copper sulphate, grains 30.
Water, fl. ounce ½.
Glycerin, fl. ounce ½.
Liquor potassæ (5 per cent. potassium hydrate), fl. ounces 5.

The great advantage of this solution is in its being always ready for use and in keeping well.

black precipitate of metallic bismuth separates. This color test is also given by albumin and many other substances. Repeat with egg albumin.

4. To some of the above solution add several drops of an alcoholic solution of ∝ -naphthol. Carefully pour some strong sulphuric acid down the side of the tube. The purplish ring formed at the junction of the two liquids is characteristic of sugar. Repeat with pure glucose.

EXPERIMENT VI.

Inorganic salts.—A part of the yeast which has been extracted with alcohol and ether for lecithin, and sodium carbonate solution for nucleins, is ignited in a porcelain crucible. The ash is treated with nitric acid, the solution diluted ten times, and an excess of ammonium molybdate solution added. This precipitates all of the phosphates. The filtrate is then neutralized with ammonia, and ammonium oxalate is added. The white crystalline precipitate shows presence of calcium. Potassium is also present, but need not be tested for.

EXPERIMENT VII.

(Enzymes in Yeast.)

Preparation of invertin.—Grind up one yeast cake with sand and water, dilute to 50 c.c., add 5 c.c. of ether, and filter clear. Add 100 c.c. of alcohol to filtrate, and filter off the precipitate. Dissolve this precipitate in water, on filter paper, add cane-sugar solution, and test for glucose after a little time. Besides the invertin, which is comparatively easy to isolate, yeast contains an enzyme, zymase, which converts sugar into alcohol and carbon dioxide, and a proteolytic enzyme which digests proteids. As these enzymes are isolated only with difficulty, they will not be further studied.

We see, then, that the yeast plant contains proteids, nucleins, carbohydrates (glycogen), fats (lecithin and cephalin), inorganic salts, and enzymes. The interaction, breaking down, and building up of these various constituents constitute the chemical side of the phenomena of life.

As the life process is represented, in part at least, by one or more chemical reactions, we find that the growth of the yeast plant follows the laws of ordinary chemical reactions.

1. The lack of the proper constituents (food supply) for the reaction will bring it to an end.

2. Accumulation of the products of the reaction causes the establishment of an equilibrium and a practical stopping of the reactions.

3. The reaction goes on quicker at higher than at a lower temperature, below the limit at which proteids are coagulated. At a higher temperature the course of the reaction may also be changed.

4. Oxygen is of great importance, the absence of oxygen changing the equilibrium of the reaction.

5. Like most chemical reactions, those of yeast go on best in solution. Removal of water or drying stops the reaction.

To illustrate each one of the above requirements, perform the following experiments.

EXPERIMENT VIII.

(To illustrate 1.)

To inoculate fermentation tubes with as nearly the same amount of yeast for comparative experiments, proceed as follows: One-fourth of a yeast cake is thoroughly stirred up with 20 c.c. of water. While constantly stirring, 1 c.c. of the mixture is taken up in a graduated pipette and transferred to a fermentation tube already filled with the required solution. The whole is then agitated, the upright tube is again filled with the liquid, and the fermentation allowed to proceed. The four tubes contain the following solutions:

	a	*b*	*c*	*d*
Glucose	......	100 g.	100 g.	100 g.
Sodium potassium tartrate	......	.....	4 g.	4 g.
Ammonium nitrate	......	.....	.....	3 g.
Sodium acid phosphate	......	.....	1 g.	1 g.
Calcium chloride	......	.....	.....	0.5 g.
Water (distilled)	1 Liter	1 L.	1 L.	1 L.

The amount of carbon dioxide collected, although the result of the action of the zymase, may be taken as an indication of the growth of the yeast plant. The solution which gives the most carbon dioxide would therefore be the one in which the conditions for the growth of the yeast are the most favorable. Roughly calibrate your tubes and tabulate observations. From your results draw conclusions as to the relative food value of the above constituents. Why are proteids not necessary? In tube *a* no gas will be found to have been collected, although the compressed yeast should contain sufficient carbohydrate to generate some carbon dioxide. Distilled water therefore acts as a poison.

EXPERIMENT IX.

(To illustrate 2.)

Fill two fermentation tubes with solution (*d*) and inoculate with the same amount of yeast. After some gas has collected, add to the tube which has the most gas 2 c.c. of alcohol (95 per cent.). Now observe rate at which the gas collects. When evolution of gas in the tube to which alcohol has been added has come to a stop, decant off the solution carefully from the yeast which has settled. Add fresh solution (*d*), decant again, add some more solution, and notice if fermentation begins again. Give an example of an ordinary chemical reaction which illustrates the law of mass-action like the above case. Repeat the above experiment, using ether instead of alcohol. Notice that the yeast will not again begin to grow. It has been killed. The alcohol, although it may, like the ether, have a physiological effect on the yeast plant, acts mainly by its mass-action in preventing the formation of more alcohol by the zymase. Considerable care must, however, be exercised in applying chemical laws to life-reactions on account of the complexity of the factors involved.

EXPERIMENT X.

(To illustrate 3.)

Inoculate four fermentation tubes containing solution (*d*) with the same amount of yeast, and grow at the following temperatures: 5° C. (in ice-box), 20° C. (at room temperature), 40° C.

(in incubator), 50° C. (in incubator). Measure amount of gas collected in each after two, three, and twenty-four hours. Test reaction to litmus of each solution after twenty-four hours. Draw conclusions. To show the effect which different temperatures will have in actually changing the character of the reaction perform the following experiments:

EXPERIMENT XI.

Take two sterilized test-tubes containing agar-agar and inoculate each with a bit of a culture of *Bacillus prodigiosus.* Place one at room temperature, the other in the incubator at 37.5°. Leave two days. The one at room temperature will be found to have developed a red pigment, while the one in the incubator produces little or none at all.

EXPERIMENT XII. (Pasteur's Experiment.)

(To illustrate 4.)

Take one-sixteenth of a compressed yeast cake and mix thoroughly in a mortar with 20 c.c. of water. Strain through cheese cloth. Add 15 c.c. of this yeast mixture to 200 c.c. of solution (*d*). Mix thoroughly and, while yeast is still uniformly suspended, divide evenly between two bottles *A* and *B*.

Cork bottle *A* with a rubber stopper having an exit tube to carry off carbon dioxide. This exit tube should end beneath the surface of water in a beaker. Divide the contents of *B* (first shaking it if the yeast has begun to settle) equally between two battery jars, thus forming a thin layer in each. Cover the jars loosely with glass plates. Set all three receptacles aside for several days. Examine both *A* and *B*, especially noting the comparative number of bubbles being given off from both. Transfer solutions from jars to bottle *B*. Shake up both bottles; take equal parts from each; centrifugalize. Compare amounts of sediment.

Set up a simple still. Successively place in the flask the entire contents of each bottle and, *using a moderate flame*, distil off exactly 15 c.c. from each. Label the distillates and cool them to the same temperature. Test the specific gravity of the distillates

by means of a small hydrometer. Note carefully any differences.

Note comparative smell and taste of distillates. State conclusions in your notebook.

EXPERIMENT XIII.

(To illustraie 4.)

Sow sterile meat-juice in two tubes with a culture of *Bacterium coli communis.* Seal one tube, leaving the other exposed to the air through the cotton plugs. Place in the incubator at 37.5°. The next morning examine each, first for smell; second, for the presence of indol as follows: Add one or two drops of strong nitric acid, and then, a drop at a time, a 2 per cent. solution of potassium nitrate. A red coloration due to nitrosoindol nitrate is produced, much stronger in *a* than in *b*.

EXPERIMENT XIV.

(To illustrate 5.)

Saturate fermentation solution (*d*) with glucose. Place in fermentation tube. In another tube place some ordinary solution (d). Inoculate with the same amount of yeast. Tabulate gas collected. Saturating the solution in the first tube with glucose has the same effect as removing the water. Draw conclusions.

If the solution were saturated with cane sugar, this effect would be even more striking. The use of cane sugar as a preservative depends upon this fact.

The effect of growing different kinds of protoplasm on the same medium is illustrated by the following interesting experiment:

EXPERIMENT XV.

Take two glucose agar tubes and inoculate one with *Bacillus coli communis,* the other with *Bacillus typhosus.* One produces gas, the other not. This is one of the diagnostic differences between the two bacteria.

CHAPTER II.

THE CHEMISTRY OF THE FOODS.

Among the primary cell constituents, studied in the previous chapter, the fats, carbohydrates, and proteids are most commonly classified as foods. Nucleic acid, lecithans, and inorganic salts are found very closely associated with these in our ordinary articles of diet. The physiological function of the three classes of foods may be summed up as follows: The proteids build up the tissues and are indispensable. The carbohydrates furnish energy, and thus save the body proteids from too rapid destruction. The fats, although they contain twice as much potential energy as either the carbohydrates or proteids, are the least essential, except when large quantities of energy are needed, as in cases of extreme cold. The fact that the fats are laid down in large part in the superficial fasciæ and are non-conductors of heat aids also in the saving of heat energy. The physical properties, chemical tests, and chemical structure, as far as determined, will be considered under each class.

FATS.

Fats are found everywhere in living matter. They occur either free, as neutral fat, or split into their components, fatty acids and glycerin, or in combination with phosphoric acid and cholin forming lecithin (see chap. 1). The acids most commonly found in fats are stearic, $CH_3(CH_2)_{16}COOH$, palmitic, $CH_3(CH_2)_{14}COOH$, and oleic, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$, although in milk fat butyric, $CH_3(CH_2)_2COOH$, caproic, $CH_3(CH_2)_4COOH$, and caprylic, $CH_3(CH_2)_6COOH$, acids are found. Instead of glycerin ($CH_2OH\text{-}CHOH\text{-}CH_2OH$) in combination with acids forming fats, we may have some other alcohol, such as cholesterin, $C_{27}H_{45}OH$, or cetyl alcohol, $CH_3(CH_2)_{14}CH_2OH$.

1. *Physical state.*—The animal fats of the higher saturated fatty acids are solid at room temperatures, but are fluid at body

temperatures. Place some mutton tallow in a test-tube containing a thermometer. Immerse the tube in water, in a beaker, and warm gradually. Observe the temperature at which the fat begins to melt. Mutton tallow is, largely, the glyceride of stearic and of palmitic acids. Repeat above experiment, using a piece of butter instead of tallow. Fats containing a large proportion of oleic acid—*i. e.,* olive oil—are fluid at room temperature.

2. *Solubility.*—Test the solubility of olive oil in water, ether, alcohol, chloroform, and in water containing a soap solution.

3. Test the reaction of olive oil to litmus paper. On standing, fats undergo a change, becoming "rancid." The reaction involved in this change is assisted by moisture and oxygen, and results in the liberation of free fatty acid.

4. *Acrolein reaction.*—Heat a small piece of tallow, in a dry test-tube, with a pinch of solid potassium bisulphate. Intensely disagreeable fumes of acrolein will be given off. This acrolein comes from the decomposition of the glycerin.

$$CH_2OH\text{-}CHOH\text{-}CH_2OH = CH_2\text{=}CH\text{-}CHO + 2H_2O.$$

5. *Saponification.*—The neutral fats, like all esters, are easily saponified or broken up into their constituents, acid and glycerine, by hydroxyl and hydrogen ions. As water contains a small number of such ions, a slight saponification may be brought about by warming fats with water. The acids and alkalies contain more of such ions and readily saponify the fats. Boil a piece of fat with a 10 per cent. solution of sodium hydrate in alcohol in a flask for one hour. To a portion of the solution add calcium chloride solution. What happens?

6. *Fatty acids.*—Evaporate off the alcohol from the rest of the above solution. Take up with water and pour the aqueous solution slowly into warm dilute sulphuric acid, stirring constantly. What is the precipitate? Filter and preserve the filtrate.

7. Wash the precipitate obtained above, with tap water and dry with filter paper. Dissolve a portion of it in hot 95 per cent. alcohol and test the reaction. Test the solubility in ether, chloroform, and hot water.

8. Neutralize the filtrate, obtained in 6, with a little sodium hydrate, evaporate almost to dryness, and extract the residue with alcohol. Filter, evaporate the alcohol on steam bath, and test residue with potassium bisulphate for acrolein. This shows presence of glycerine.

CARBOHYDRATES.

The carbohydrates are found, for the most part, in vegetable tissues; yet some are found in animal tissues. They are composed of carbon, hydrogen, and oxygen, the two last usually in the proportion to form water, but, occasionally, as in rhamnose, this proportion does not hold. The monosaccharides are either aldehydes or ketones. The carbohydrates are divided into three classes—the monosaccharides, the disaccharides, and the polysaccharides. A constituent member or two of each group will be studied:

1. *Monosaccharides.*

a) *Dextrose* (d-glucose), $CH_2OH\text{-}(CHOH)_4\text{-}CHO$. Test its solubility in water, in alcohol, and in ether. Apply the tests given for carbohydrates in chap. 1. Apply the reduction test with Barfoed's reagent (13 g. of cupric acetate dissolved in 200 c.c. of water, and 5 c.c. of 38 per cent. acetic acid added.)

b) *Fructose* (lævulose), $(CH_2OH)(CHOH)_3CO\text{-}CH_2OH$. Test its solubility in the various menstrua. Apply the tests given in the former chapter for carbohydrates. Note any variations in reactions of lævulose from those of glucose.

Seliwanoff's test: Boil some lævulose with a solution of resorcin in very dilute hydrochloric acid. Note red coloration of solution and dark-red precipitate on standing. This precipitate is soluble in alcohol with dark-red coloration. This test is given only by lævulose and sorbose. Apply Barfoed's reduction test.

2. *Disaccharides.*

a) *Cane sugar,* $C_{12}H_{22}O_{11}$. Test its solubility in water, alcohol, and ether. Apply the reduction tests for carbohydrates, and notice variations from the monosaccharide reactions.

Dissolve some of the cane sugar in water and boil with a few drops of concentrated hydrochloric acid. Now apply above reduction tests and observe results.

Cane sugar is split up by action of acids or alkalies into glucose and lævulose. Show presence of each in the solution obtained by action of dilute hydrochloric acid upon cane sugar.

Boil some of the cane-sugar solution with an equal volume of concentrated hydrochloric acid. Notice formation of deep-red color. The monosaccharides or other disaccharides do not give this latter reaction.

b) *Lactose,* $C_{12}H_{22}O_{11}$. Test its solubility. Apply the reduction tests cited above. Add a few drops of concentrated hydrochloric acid to a lactose solution and boil. Apply the test with Haines's and Barfoed's solutions. Notice variation from the results obtained before treatment with hydrochloric acid.

c) *Maltose,* $C_{12}H_{22}O_{11}$. Apply tests given under *Lactose,* and observe variations.

d) Determine angle of rotation of equimolecular solutions of maltose, cane sugar, lævulose, and glucose with polariscope.

3. *Polysaccharides.*

a) *Starch* ($C_6H_{10}O_5$). Test its solubility. Grind up a little starch with cold water in a mortar, pour into a beaker of boiling water, notice the formation of an opalescent solution. Now add a few drops of solution of iodine in potassium iodide and observe the blue coloration. Heat the mixture and observe the result. The blue coloration of starch with iodine is due to the formation of the iodide of starch.

To some starch solution add a few drops of concentrated hydrochloric acid and boil for a few minutes. Test the resulting solution for glucose and dextrin (as given below).

b) *Dextrin* ($C_6H_{10}O_5$). Test solubility of dextrin in water, alcohol, and ether. Add to solution of a dextrin a few drops of iodine solution and notice the formation of mahogany-red color.

c) *Glycogen* ($C_6H_{10}O_5$). See preparation under "Liver" (chap. 4).

PROTEIDS.

The proteids are among the most important constituents of the living cell. They are amorphous bodies of large molecular weight and are built up of carbon, hydrogen, oxygen, nitrogen, sulphur, and, sometimes, iron and phosphorus. Their structure has not been as yet determined with any degree of accuracy. The splitting products, which throw some light on their structure, will be considered after we have studied the classification of the proteids by preparing a characteristic member of each group.

The following classification is based on the work of Hoppe-Seyler, Drechsel, Kossel, Hammarsten, and others.

CLASSIFICATION OF PROTEIDS.

A. *Simple proteids* (albumins).
  - *a*) *Native albumins:* serum albumin, egg albumin, lact albumin, histon.
  - *b*) *Native globulins:* serum-globulin, fibrinogen, myosinogen, cytoglobulin, globin (from hæmoglobin), vegetable proteids (edestin).
  - *c*) *Denaturalized* (*derived*) *albumins.* enzyme coagulated: fibrin, myosin: heat coagulated; acid treatment: acid albumin (syntonin); alkali treatment: alkali albumin.
  - *d*) *Albuminoids* (supposed by some to have been derived from the proteids of the cell by chemical change): collagen (from bone), elastin (from fibrous elastic tissue), keratin (from horn), reticulin, fibroin (from silk), protamin (from fish sperm).

B. *Compound proteids* (simple proteid + R).
(albumin + R).
  - *a*) Nucleo-proteids—nucleic acid and albumin.
  - *b*) Phosphorized albumins—paranucleic acid and albumin (casein) (nucleo-albumins).
  - *c*) Chromoproteids—chromophore group and albumin (oxyhæmoglobin).
  - *d*) Lecitho-albumins—lecithin and albumin (vitellin).
  - *e*) Amyloid—chondroitin sulphuric acid and albumin.
  - *f*) Glycoproteids—sugar and albumin (mucin, which also contains chondroitin sulphuric acid).

One or two characteristic members of each of the above groups are to be prepared and tested as follows:

Physical properties: Solubility in water and sodium chloride solution. Temperature of coagulation.

Chemical tests: Proteid reactions. Test for sulphur and phosphorus.

The main bulk of each preparation, not used in the above tests, must be carefully dried preferably on a porous porcelain plate, without the use of heat, and handed to the instructor in a labeled sample tube.

Preparations I and II.

I. *Serum-globulin.*—Obtain some blood serum from the instructor. This serum has been made by coagulating the fibrinogen as fibrin and centrifugalizing to remove the corpuscles. Observe color, odor, consistency, and reaction of this serum. Heat a little of this serum in a test-tube; notice coagulation and temperature at which coagulation takes place. To another portion (5 c.c.) add 200 c.c. of distilled water. What is the precipitate? Filter and heat the filtrate. What are your conclusions from above observations?

The main bulk of the serum is saturated with magnesium sulphate at 30° C. Stir well and filter. What is the precipitate? Preserve the filtrate for preparation of serum-albumin and proceed with the precipitate as follows: Wash the precipitate with water. Owing to the admixture of magnesium sulphate with the globulin, this latter will gradually go into solution as the washing proceeds. To get rid of the salts and prepare the pure globulin, place this solution (of globulin in dilute salt solution) in a dialyzing tube of parchment paper, and allow it to dialyze for forty-eight hours, changing the water on the outside frequently. At the end of dialysis remove the globulin into a large bulk of distilled water, allow to settle, filter, and dry the precipitate on a porcelain plate.

II. *Serum albumin.*—The filtrate, obtained above after saturating the serum with magnesium sulphate and filtering off the precipitated globulin, is acidified with acetic acid until the resulting solution contains 1 per cent. acid. Notice the heavy precipitate formed. Filter, dialyze the precipitate against distilled water for forty-eight hours, and dry the precipitate. Note variations in chemical and physical characteristics of this latter precipitate as compared with those of globulin..

Preparation III: Vegetable proteid (Edestin, Osborn's method).—One hundred g. of hemp-seed meal are extracted at 60° for six hours with 300 c.c. of 4 per cent. sodium chloride. Add enough baryta water to neutralize the extract and strain through muslin. Filter this strained extract through thick felts of filter pulp until the filtrate is perfectly clear. Place this clear filtrate in a flask, cork, and allow to stand in ice-box over night. The edestin, which separates out on standing, is then recrystallized by dissolving in 8 per cent. sodium chloride solution, heating to 50°, diluted with two volumes of water, and allowed to cool over night. This may be filtered off, washed with alcohol at 0°, and dried over sulphuric acid. Test its solubility in water, alkalies, and acids.

Preparation IV· Acid albumin (syntonin).—Take 100 g. of lean meat, finely divide with meat-chopper, and heat to boiling with 200 c.c. of 10 per cent. hydrochloric acid. After the solution has boiled, allow it to stand for one hour. Filter through muslin and then through filter paper. Exactly neutralize the filtrate with 10 per cent. sodium hydrate solution. Notice the precipitate which appears at once or on standing for a few minutes. Allow this precipitate to settle, decant off the clear fluid, and pour the precipitate upon a smooth filter. Redissolve the precipitate in dilute hydrochloric acid, filter from undissolved residue (which should be very slight, if any), and reprecipitate the syntonin from the filtrate by neutralization with dilute sodium hydrate. Place the reprecipitated syntonin on a porcelain plate to dry.

Preparation V: Collagen.—Carefully clean a small piece (about 20 g.) of bone from adherent muscle and tendon. Place it in a beaker with 100 c.c. of 5 per cent. hydrochloric acid, and allow it to stand two to three days. The inorganic constituents dissolve in the acid, leaving the organic matter. Note the effervescence due to the presence of the carbonates. Examine the elasticity of this decalcified bone. Pour off the acid solution and

keep it for future tests for inorganic constituents. Place the decalcified bone in a 10 per cent. solution of sodium carbonate and allow it to stand over night. Pour off the sodium carbonate, bring the bone into 100 c.c. of water, and boil for an hour or two. By boiling the ossein or callagen is converted by hydration into gelatine. Evaporate the solution on the water-bath to about 30 c.c. Filter and set the filtrate aside to cool. On cooling it should "set" or "gel." If it does not, concentrate further. Hand in a specimen of this gelatine. With the remainder proceed as follows: Redissolve the gelatine by adding an equal quantity of water and heating. With this solution try the following tests: (1) Biuret test. (2) Millon's test. (Upon what chemical group does Millon's test depend? If given by gelatine an impurity is present.) (3) Potassium ferro-cyanide and acetic acid. (4) Tannic acid. (5) Basic lead acetate (if this reaction is negative, explain result.)

Preparation VI: Phosphorized albumin (nucleo-albumin; casein).—Dilute 20 c.c. of milk, as free as possible from cream, with 500 c.c. of distilled water. Add gradually, stirring constantly, dilute acetic acid until the solution becomes slightly acid. Note the heavy flocculent precipitate of casein. Filter off this precipitate, and test the filtrate for albumin and lactose. Redissolve this precipitate in 50 c.c. of 5 per cent. sodium carbonate solution. Some of the precipitate will remain undissolved owing to admixture with fatty matter. Filter, dilute the filtrate with 250 c.c. of water and reprecipitate the casein by addition of glacial acetic acid, drop by drop. Dry the precipitate on porcelain plate, hand in a sample, and test the remainder as follows: (1) Albumin tests. (2) Fuse a portion with fusion mixture and test for phosphorus.

Preparation VII: Glycoproteid (mucin).—To 100 c.c. of saliva (collected before a meal) add dilute hydrochloric acid, until the solution contains 1.5 per cent. hydrochloric acid. Notice the formation of stringy flakes of mucin, which redissolve when the acidity reaches the point indicated. Dilute the solution at once with four times the amount of distilled water. Mucin is precipitated. Filter, redissolve the precipitate with dilute hydrochloric acid, and reprecipitate with water. Dry precipitate and hand in a specimen. With the remainder make following tests: (1) Test the precipitate with Haines's or Fehling's solution for sugar. Note that no reduction of the copper solution occurs. (2) Boil some of the mucin with dilute hydrochloric acid for a few minutes, and then test the solution for sugar. Observe reduction. Mucin is a glycoproteid and, on boiling with acid, splits off glucose. This test may also be shown by boiling the saliva with dilute hydrochloric acid and testing for sugar.

As the proteids are broken up by steam, acids, or enzymes, they first split into non-crystalline substances of large molecular weight, which do not throw any particular light on the structure, but are nevertheless important in the study of digestion. Among these decomposition products may be mentioned proto-, hetero-, and deutero-albumoses, and peptones.

Obtain from the instructor 20 g. of commercial peptone (10 g. American peptone + 10 g. Witte's). Dissolve this in 70 c.c. of distilled water and neutralize with sodium hydrate or hydrochloric acid, according to reaction of original solution. Notice the odor, taste, color, and reaction of the solution. Filter if the solution is not clear.

Preparation VIII: Protalbumose, hetero-albumose (primary albumoses).—To the 70 c.c. of solution add 70 c.c. of a *saturated* ammonium sulphate solution and stir. The sticky precipitate remains for the most part attached to the beaker. Collect the precipitate on a rod by stirring; filter. Save the filtrate.

Tests for protalbumose.—1. Dissolve the precipitate of proto-albumose in a little water. To a portion of this solution add an excess of sodium hydrate and a little copper sulphate solution. Observe the purple, red, or violet biuret reaction. Save this tube and compare with the biuret color of the deutero-albumoses and peptone.

2. To a portion of the solution of proto-albumose add enough acetic acid to make it slightly acid and then a few drops of potassium ferro-cyanide. A precipitate appears. Is it soluble on heating?

3. To a third portion of the solution add a little copper sulphate solution. A white precipitate of the copper compound of the proto-albumose is thrown down.

4. To a fourth portion add a little nitric acid. Notice the precipitate. This dissolves on warming.

Preparation IX: The deutero-albumoses (secondary albumoses).—To the filtrate from the protalbumoses add powdered ammonium sulphate as long as the sulphate redissolves, shaking thoroughly with each addition. As the saturation of the solution with ammonium sulphate proceeds, hetero-albumose separates out, but may be disregarded. The solution may be concentrated by evaporation. Then add to the solution 2 c.c. of a saturated ammonium sulphate solution slightly acid with sulphuric acid (five drops of concentrated acid to 10 c.c. of the saturated solution). The precipitation is nearly complete. Filter the precipitate which contains the deutero-albumoses. Save the filtrate for the peptone.

Test for the deutero-albumoses.—Repeat tests for the protalbumoses with copper sulphate, nitric acid, and potassium ferrocyanide. No precipitates appear. Compare the biuret color with that of the filtrate (peptone) and the proto-albumoses. If the admixture of ammonium sulphate be very great, the color of the biuret reaction will be a deep-blue. A large excess of sodium hydrate must be added to obtain the true biuret color.

Preparation X: Peptone.—Concentrate the filtrate to half its volume on the water-bath, filter from albumoses which have separated out by concentration, and then place filtrate outdoors to cool. Filter off the crystals of ammonium sulphate. Dilute the filtrate with an equal volume of water and add barium hydrate in slight excess. Heat to boiling, filter hot from barium sulphate, and neutralize the excess of barium in the filtrate with dilute sulphuric acid. Filter the hot solution, and concentrate the filtrate to 25 or 30 c.c. on the water-bath. Test the evaporated filtrate with the biuret test. Pour the remainder into twice its bulk of 90 per cent. alcohol and allow to stand until the precipitate (which consists, for the most part, of inorganic salts, but contains some peptone which is insoluble in the alcohol used) settles out. Pour off the clear fluid and evaporate it nearly to dryness. Dry on porcelain plate. Hand in sample and dissolve a portion of the yellow, sticky peptone in a little water, and repeat the above tests for the albumoses.

The final splitting of proteids which leads to crystalline decomposition products of known chemical structure, such as tyrosin, leucin, di-amino acids, cystein, and certain mono-amino dibasic acids, such as glutaminic and aspartic acids, can be best illustrated by the following experiment.

SPLITTING PRODUCTS OF THE PROTEIDS.

The final splitting or cleavage of proteids may be brought about by the action of superheated steam, of acids, of alkalies, or of enzymes. In the following experiment advantage is taken of the fact that proteids, if submitted to the action of strong acid for a few hours at boiling temperature, will split up into their ultimate cleavage products, which may be isolated and detected.

Preparations XI, XII, and XIII.

1. Take 100 c.c. of solution, prepared by instructor as follows: Take 100 g. of ordinary blood fibrin and heat to boiling in a flask with 250 c.c. of 20 per cent. hydrochloric acid for twelve hours, using a reflux-condenser to prevent loss by evaporation. After the mixture has cooled, filter off the brownish or deep-black liquid. Note the black residue on the filter and sides of the flask. This is known as "humin" material or melanoidinic acid.

2. Take the filtrate from above and decolorize by boiling with 15 g. of animal charcoal for ten minutes. Filter. The filtrate should be nearly colorless or faintly yellowish.

3. Evaporate this filtrate, on water-bath under the hood, nearly to dryness, in order to drive off the hydrochloric acid used in splitting the proteid. Dissolve the residue in 100 c.c. of hot water, filter, if necessary, and proceed with the filtrate as follows:

4. Take 10 c.c. of the filtrate and neutralize it with sodium hydrate. Notice the abundant grayish-white precipitate. This

is commonly called acid albumin; it is peculiar, however, in that it does not dissolve in alkalies. Test it for proteid reactions.

5. Filter off this "acid albumin," and test filtrate for albumoses and peptone according to directions given earlier in this chapter.

6. Take 2 c.c. of the filtrate obtained in 3, and test for tryptophan (skatol-amino acetic acid) as follows: Add to the solution (which should be only very slightly acid) a little bromine water. Note the violet coloration which forms after a short time.

7. Take 10 c.c. of filtrate obtained in 3 and add phosphotungstic acid solution (2 per cent.) as long as a precipitate forms. Filter off this precipitate (which consists of the di-amino acids or hexon bases, arginin, histidin, and lysin, together with any peptone, albumoses, and xanthin bases which may be present). Dry this precipitate on porcelain plate and hand in as hexon bases (Preparation XI). These bases may be separated (best by the Kossel-Kutscher method) and isolated as distinct compounds.

8. Evaporate the main bulk of the filtrate obtained in 3 to dryness on water-bath. Treat dry residue with 50 c.c. of cold water. The salts (principally sodium chloride) and leucin go into solution, while the tyrosin, owing to its sparing solubility in cold water, will largely remain insoluble. Filter. Save residue for future work on tyrosin and proceed with filtrate as follows:

9. Evaporate the filtrate from above to dryness. Take up the residue with 50 c.c. of 95 per cent. alcohol. The leucin goes into solution, while the salts, for the most part, remain insoluble. Filter and evaporate the alcoholic filtrate to a syrup and allow the leucin to crystallize out. Dry the crystals on a porcelain plate. Hand in a sample (Preparation XII) and test the remainder as follows:

a) Evaporate some of the crystals with concentrated nitric acid on a platinum foil, warm the colorless residue with a few drops of sodium hydrate solution, and note the color ranging from yellow to brown. If further heating be applied, the leucin will collect into an oily drop (Scherer's test).

b) Add ammonium hydroxide to a few c.c. of lead acetate solution. Filter off the precipitate of lead hydrate and add the filtrate to a little (5 c.c.) of aqueous solution of leucin. Boil the mixture and note the precipitation of white crystalline plates of leucin lead oxide which form in a short time.

c) Examine the crystals of leucin microscopically. Note hyaline balls or spherules with alternating light and dark concentric layers.

10. The residue obtained in 8 is dried on porcelain plate. Hand in specimen of this as tyrosin (Preparation XIII) and test remainder as follows:

a) *Hoffman's test.*—Add to the hot-water solution of the crystals a few drops of Millon's reagent. Heat and observe the deep-red coloration.

b) *Piria's test.*—Dissolve some of the crystals in hot concentrated sulphuric acid. Cool, dilute with water, neutralize with barium carbonate, and filter. On addition of a few drops of ferric chloride solution to the filtrate, a violet coloration is observed.

c) *Mörner's test.*—Add to an aqueous solution of tyrosin a little (1 c.c.) of reagent made up as follows: Formalin, 1 c.c.; concentrated sulphuric acid, 55 c.c.; water, 45 c.c. Heat the mixture and notice the green color which appears after boiling.

d) Examine some of the crystals microscopically. Note the needle-shaped crystals, often arranged in sheaf-like formations.

Leucin and tyrosin may also be separated by use of boiling glacial acetic acid, which dissolves the leucin, leaving the tyrosin as an insoluble residue.

Preparation XIV: Cystin (Embden's method).—Take 500 g. of horn and treat with 1500 c.c. of concentrated hydrochloric acid; heat to boiling, with reflux condenser, for five to six hours. Filter and neutralize the fluid with strong sodium hydrate (avoid heating). Allow this to stand twenty-four hours, filter, weakly acidify the filtrate with hydrochloric acid, and decolorize it with animal charcoal. Allow to stand twenty-four hours. Tyrosin with some cystin may separate out. If so, filter and evaporate filtrate to crystallization. Repeat evaporation and filtration from crystals until about 100 c.c. of fluid remains. Treat the crystal-

lization fractions as follows: Treat crystals with some cold water; sodium chloride and leucin go into solution. (The leucin may be separated from the salt by evaporation of the solution to dryness and dissolving residue in alcohol, the salt remains insoluble.) Dissolve the residue, insoluble in cold water, in a few cubic centimeters of ammonium hydroxide, filter, and allow the ammonia to evaporate spontaneously, or acidify the ammoniacal solution with acetic acid; tyrosin and cystin separate out and should be washed with water. Treat the mixture of cystin and tyrosin with cold water (100 c.c. for every 5 g. of precipitate), add dilute sulphuric acid, drop by drop. The tyrosin goes into solution. Filter, wash residue of cystin with cold water, dissolve in ammonium hydrate, and recrystallize as above. Hand in sample and test remainder as follows:

a) Take some of the cystin crystals; add a few c.c. (2) of sodium hydrate solution and a few drops of lead acetate solution. Heat the mixture, and notice the heavy black precipitate of sulphide of lead.

b) Dissolve some of the crystals in strong sodium hydrate solution, add a few drops of benzoyl chloride, and shake vigorously. Note the voluminous precipitate of benzoyl-cystin.

c) Treat some of the crystals with a little granulated zinc and add dilute hydrochloric acid. Note the slight production of hydrogen sulphide. Add to this solution (after evolution of hydrogen) a cubic centimeter of mercuric chloride solution. Notice the heavy precipitate of cystein mercuric chloride.

Detection of sulphur in proteids.—Sulphur exists in proteids in two forms—the loosely combined (unoxidized) and the firmly combined (oxidized).

The loosely combined is usually referable to cystin and is detected as in (*a*) under cystin.

The firmly combined sulphur is detected as follows: Mix some egg albumin with fusion mixture and warm cautiously in a porcelain crucible until the mixture is nearly colorless. Dissolve fused mixture in a few cubic centimeters of dilute hydrochloric acid, filter, and treat the warm filtrate with barium chloride solution. Observe the precipitate of barium sulphate.

From the experiments outlined in this chapter it will be seen that syntonin, albumoses, peptone, hexon-bases, leucin, tyrosin, cystin, tryptophan, and other substances are formed in the hydrolytic cleavage of proteids. These same substances are formed in the body during digestion and are utilized by the body in performing certain of its characteristic metabolic processes. A study of the action of the digestive juices upon foods will next be taken up. It is to be noted, in the following exercises, that the action of a digestive ferment is simply an accelerating one, hastening the process which under the proper conditions may be brought about solely by the hydrogen or hydroxyl ions present in the digestive fluids.

CHAPTER III.

THE CHEMISTRY OF DIGESTION.

The fats, carbohydrates, and proteids, in order to be of value to the organism as foods, must as a rule be broken up into their ultimate splitting products (studied in the previous chapter), or at least into smaller molecules. This is accomplished by means of fluids (containing ferments) secreted into the digestive tract from a number of glands situated at various points. The object of this splitting up is to render the absorption of the foods more rapid and complete, and also to enable the organism to build up out of food-stuffs of varying composition its own constituents, which change very little in their characteristics, no matter how varied the diet. The fats, under abnormal conditions, are an exception. The nature of the various secretions and their action on the foods in their passage through the digestive tract will be studied in the order in which they are met.

SALIVARY DIGESTION.

I. *Composition and action of saliva.*—The saliva is the combined secretion of the various glands in the cavity of the mouth. These glands—the parotid, submaxillary, sublingual, and buccal—produce secretions differing the one from the other in chemical composition and properties. The mixed saliva is a liquid of variable composition, depending on conditions which precede the secretion.

Collect some saliva by chewing a small quantity of paraffin and expectorating into a test-tube.

1. Examine properties—color, consistency, odor, and reaction. On allowing the saliva to stand for some time a distinct turbidity will be noticed. This is due to the precipitation of carbonate of calcium, previously held in solution by the carbon dioxide, which has now escaped into the air.

2. Filter some fresh saliva and apply the following tests to the filtrate: (*a*) Millon's, (*b*) biuret, (*c*) xanthoproteic, (*d*) Haines's.

3. The inorganic constituents of saliva are essentially those found in all protoplasm.

4. Besides the above constituents of saliva, another, the sulphocyanate of potassium (KCNS) is present in appreciable quantity. This may be detected as follows:

a) Add to a few cubic centimeters of filtered saliva, collected before meals (breakfast), a few drops of hydrochloric acid, and then a drop or two of ferric chloride solution. Notice formation of red color. Observe action of mercuric chloride upon this colored solution.

b) Treat some saliva with one or two drops of dilute hydriodic acid and then add a little starch flour; the saliva becomes blue. This reaction shows the presence of a sulpho-cyanate, as this is the only substance found in saliva which will reduce the hydriodic acid. This potassium sulpho-cyanate is secreted by the parotid gland only.

5. *Action of saliva.*—Put some 0.5 per cent. starch solution (directions, chap. 2) into four test-tubes. Add some filtered saliva to *A*. To *B* add saliva and two or three drops of hydrochloric acid (concentrated); to *C* add saliva and 1 c.c. of sodium hydrate solution; to *D* add saliva and boil. Put all the tubes in water-bath at 40° for 15 minutes. Test the various solutions for sugar. Conclusions from these experiments? Always test the starch and saliva separately for sugar before trying the action of ptyalin on starch. If *D* reduces Haines's solution, the sugar is derived from the mucin.

We see from the above experiments that saliva is a mixture of organic and inorganic substances. The organic substances are mucin (see chap. 2), albumin, ptyalin, which in slightly alkaline solution splits starch into maltose and glucose, and potassium sulpho-cyanate. The inorganic substances are sodium chloride, potassium chloride, sodium carbonate, calcium phosphate, calcium carbonate, and magnesium phosphate.

II. *Action of salivary ferment ptyalin.*—To 10 c.c. of starch solution warmed to 40° C. add 1 c.c. of filtered saliva. Note an almost instant clearing of the mixture. To a few cubic centimeters of the mixture add a drop of iodine in potassium iodide solution. Note the brown or reddish color produced. This shows the first part of the action of the ptyalin. The starch has been converted first into soluble starch (amylodextrin) and then into the erythrodextrin.

1. Allow the saliva to act upon the starch solution at 40° for a few minutes more. Test with iodine solution. Note loss of color. Test this solution for reducing sugar. By this further action the process of hydrolysis has been carried to the final stage, in which the formation of acroo-dextrin, maltose, and a small amount of glucose is noted.

2. To 10 c.c. of starch solution add saliva and place the tube in the water-bath at 40°. Test the rapidity of the diastatic action of saliva by the amount of Haines's solution reduced.

3. The action of ptyalin, like that of all ferments, is greatly influenced by the presence of inorganic salts. Make two sets of experiments, testing in one case for erythrodextrin with iodine, and in the other case for glucose with Haines's solution. Use in each case the same amount of starch. Take six test-tubes for each series and 10 c.c. starch solution, then add

To tube *A*, 1 c.c. of 2 per cent. sodium chloride solution.
To tube *B*, 1 c.c. of 3 per cent. potassium chloride solution.
To tube *C*, 1 c.c. of 2 per cent. ammonium chloride solution.
To tube *D*, 1 c.c. of 2 per cent. sodium fluoride solution.
To tube *E*, 1 c.c. of 6 per cent. sodium sulphate solution.
To tube *F*, 1 c.c. of water.

Place these tubes in water-bath at 37.5° C., add 2 c.c. filtered saliva to each, and test rate of action. Tabulate results and draw conclusions.

4. *Isolation of ptyalin.*—Take 20 c.c. of filtered saliva and add to this 150 c.c. of alcohol. Note the precipitate formed. Filter and test the amylolytic power of the precipitate.

5. Examine the properties of the precipitate. Does it give the albumin reactions? Test action of salts of heavy metals on watery solution of ptyalin.

GASTRIC DIGESTION.

I. COMPOSITION AND ACTION OF GASTRIC JUICE.

1. Examine the gastric juice collected from a dog by a stomach fistula, for odor, clearness, reaction, albumin, glucose, chlorides, and phosphates.

2. *Digestive action.*—*a*) Place 5 c.c. of the juice in a test-tube, add a small piece of fibrin, place in the water-bath at 38° C., and determine the time required for its solution.

b) Add 2 c.c. of the juice to a solution of cane sugar, which gives no reaction with Haines's solution. Place in the water-bath for one hour. Remove and test for glucose. What has taken place?

c) Add a small piece of neutral butter to the gastric juice, leave for 1 hour at 40° C., and test by odor for butyric acid (action very slight).

d) Boil a little juice first, and repeat the above experiments.

e) Carefully neutralize 5 c.c. of the gastric juice, add milk, and place in water-bath at 40° C. Notice clotting. Why must the juice be neutralized for this experiment?

We see from the above experiments that normal gastric juice consists of a mixture of organic and inorganic substances, has an acid reaction, digests fibrin readily in acid solution, and clots milk in neutral solution. There is also a slight action on cane sugar. For a more careful study of these various reactions, artificial gastric juice and a neutral solution of pepsin must be used.

Artificial gastric juice may be prepared as follows: Make up an acid solution consisting of 1-10 per cent. hydrochloric acid, 1-20 per cent. lactic acid, and 1-40 per cent. butyric acid, and 1-40 per cent. acetic acid. Dissolve in each liter of this solution 2 g. of Armour's pepsin and 1 g. of rennin. Add a trace of sodium phosphate and chloride, a little albumin, and a trace of glucose.

II. ACTION OF PEPSIN, RENNIN, AND HYDROCHLORIC ACID.

1. *Action on sugar.*—Gastric juice inverts sugars, as determined in experiment. This inversion might be due to a ferment or to the hydrogen ions of the acid. Careful determinations indicate that the speed of inversion is about the same as that of an equal strength of hydrochloric acid, thus indicating that the inversion is not due to ferment action. Place 10 c.c. of 1 per cent. cane-sugar solution in each of two test-tubes. To one add 3 c.c. of 0.2 per cent. hydrochloric acid, to the other add 3 c.c. neutral pepsin dissolved in 0.2 per cent. hydrochloric acid. Warm at 40° C., and test small portions of each every five minutes for glucose. Usually the hydrochloric acid alone yields more reducing sugar than the tube containing the pepsin, as a little hydrochloric acid may be combined with the neutral pepsin. The amount of inversion of carbohydrates in the stomach depends therefore on the number of free hydrogen ions, and is only very slight under normal conditions.

2. *Action on milk.*—Gastric juice clots milk before digesting it. This is due to the presence of the ferment rennin. Calcium ions must be present to assist in forming the clot. Perform the following experiments: Place 10 c.c. of milk in each of three test-tubes. To tube *A* add 3 c.c. of neutralized artificial gastric juice containing rennin; to tube *B* add 3 c.c. of the neutralized juice which has been boiled first; to tube *C* add a few cubic centimeters of a dilute ammonium oxalate solution, and then 3 c.c. of the neutralized juice. Place all three in the water-bath at 40° C. for fifteen to twenty minutes. Examine tubes and record results. Then add two to three drops of calcium chloride solution to the third. The liquid clots. Why? Distinguish between clotting and precipitation.

3. *Action on proteids.*—The action of gastric juice on proteids is dependent on the presence of two factors, pepsin and free hydrochloric acid.

a) To distinguish the relative importance of these factors, place small pieces of fibrin in small test-tubes, and label them 1, 2, 3, 4, and 5.

To 1 add 3 c.c. of neutral pepsin solution and 10 c.c. of water.

To 2 add 10 c.c. of 0.2 per cent. hydrochloric acid solution.

To 3 add 3 c.c. of neutral pepsin and 10 c.c. of 0.2 per cent. hydrochloric acid.

To 4 add 3 c.c. of neutral pepsin and 10 c.c. of 0.2 per cent. sodium carbonate solution.

To 5 add 3 c.c. of neutral pepsin and 10 c.c. of concentrated hydrochloric acid.

Place the test-tubes in a water-bath at 40° C. and examine one hour later. Leave until next day. Notice amount digested. Filter. Apply biuret test. Compare color. Make control with pepsin alone for biuret test. Compare intensity and colors of biuret, to determine amount of digestion.

b) Place a few shreds of fibrin in 10 per cent. hydrochloric acid and boil for twenty minutes. Filter and test the filtrate for the biuret reaction. It will be found that some of the fibrin has been digested. This experiment indicates that we are to regard the acid of the gastric juice as the digestive agent, and pepsin as a substance which accelerates that action at body temperature.

c) The digestive powers of the acids are proportional to their dissociation or content of hydrogen ions and are only partly independent of the anion. To test this, place in separate test-tubes 10 c.c. each of 1-10 *n.* hydrochloric, sulphuric, nitric, acetic, oxalic, phosphoric, and lactic acids. Add to each 3 c.c. neutral pepsin solution, then add equal sized pieces of fibrin. Observe the speed of digestion in each and arrange in your notebooks the acids in order of speed of digestion. Confirm by biuret test in filtered solution.

d) Compare the action of a vegetable ferment (papain) with that of the animal pepsin.

e) *The result of the action of pepsin and hydrochloric acid on proteids.*—Place about 5 g. of fibrin in 100 c.c. of the artificial gastric juice in a stoppered flask, and allow it to remain in the warm closet at 40° C. for one hour. Filter a portion from undigested residue and place remainder in warm closet. (What is this residue?) Neutralize this filtrate carefully and exactly with 10 per cent. sodium hydrate, and filter. What is the precipitate?

Test its solubilities in acids and alkalies. After digestion of the remainder over night, filter. The filtrate contains the more highly digested proteid products. To obtain these, add to the filtrate an equal amount of a saturated ammonium sulphate solution. The protalbumoses, hetero-albumoses, and dysalbumoses separate out. These will stick to the side of the beaker for the most part. Filter. Subject the precipitate to the tests described for protalbumose.

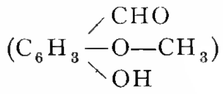
The filtrate contains deutero-albumose and any peptone. Add to the filtrate solid ammonium sulphate until it is just saturated, then add to it a small amount (1 c.c.) of a saturated ammonium sulphate solution slightly acid with sulphuric acid (five drops of concentrated acid to 10 c.c. of the ammonium sulphate solution). The precipitate is deutero-albumose. Test it by the reactions given above. Filter and show presence of peptone in filtrate by method given in chap. 2. If gastric digestion continues long enough, crystalline products will be obtained.

III. TESTS FOR FREE HYDROCHLORIC ACID.

A number of tests have been devised for the detection of free hydrochloric acid in the stomach and its differentiation from lactic and acetic acids. Hydrochloric acid appears to be more efficient in digestion than lactic acid, owing only to its greater degree of dissociation. What ought to be measured is the amount of hydrogen ions which gastric juice contains, since this is the important factor in the efficiency of the juice. This can be determined directly by measuring the speed of any catalytic reactions due to hydrogen ions. The most convenient reaction to use is the speed of inversion of cane sugar. Several indirect methods have been devised for determining the presence of free hydrochloric acid as distinguished from lactic or combined hydrochloric acid or acid phosphates. These methods are only approximately true and must be used with discretion.

1. *Töpfer's test:* Dimethyl-amido-azobenzol ($N(CH_3)_2C_6H_4\text{-}N{=}N\text{-}C_6H_5$).—Use one or two drops of a 0.5 per cent. alcoholic solution. In the presence of free mineral acids a car-

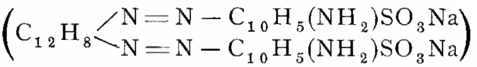
mine-red color is obtained. This test depends upon the fact that the mineral acids are more dissociated than organic acids. Place in three test-tubes 1 c.c. of *n*-10 hydrochloric acid, 1 c.c. *n*-10 lactic acid, and 1 c.c. *n*-10 acetic acid respectively. Add to each tube one drop of the reagent. Notice the difference in colors.

2. *Gunzburg's reagent:* Phloroglucin ($C_6H_3(OH)_3$) 2 g., vanillin  $(C_6H_3\begin{array}{l}\diagup CHO\\ -O-CH_3\\ \diagdown OH\end{array})$ 1 g., alcohol 100 c.c.—Place two or three drops of the solution to be tested in a porcelain dish, add one or two drops of the reagent, and evaporate on a water-bath. In the presence of free mineral acid a rose-red color is developed.

3. *Boas's reagent:* 5 g. of resorcin ($C_6H_4(OH)_2$) and 3 g. of cane sugar ($C_{12}H_{22}O_{11}$) dissolved in 100 g. of 94 per cent. alcohol.—Treat gastric juice as with Gunzburg's test. A rose-red color appears, which gradually fades on cooling, in presence of mineral acids.

4. *Tropæolin OO:* ($C_6H_4\,(SO_3H)-N=N-C_6H_4-NH\,C_6H_5$)—. Use one or two drops of a saturated alcoholic solution. Add same amount of gastric juice, and heat gently in evaporating-dish. A beautiful blue color appears in presence of all acids.

5. Test reaction with congo-red.



$$\left(C_{12}H_8\begin{array}{l}\diagup N=N-C_{10}H_5(NH_2)SO_3Na\\ \diagdown N=N-C_{10}H_5(NH_2)SO_3Na\end{array}\right)$$

Add a drop or two of solution of congo-red to same amount of gastric juice. Notice beautiful blue color, which appears in presence of free acids only.

IV. TESTS FOR LACTIC (CH_3-CHOH-COOH), BUTYRIC (CH_3-CH_2-CH_2-COOH), AND ACETIC ACIDS (CH_3-COOH).

About 20 c.c. of filtered gastric juice are extracted with 50 c.c. of ether, shaking frequently, for twenty minutes. Pipette off the ethereal extract, evaporate to dryness on the water-bath,

and take up residue with 25 c.c. of distilled water. Test separate portions of this solution for lactic, butyric, and acetic acid as follows:

1. *Lactic acid.*

a) *Uffelmann's test.*—Make up Uffelmann's reagent as follows: To a few drops of carbolic acid solution add a few drops of ferric chloride solution, and dilute this deep-blue mixture with water until a light-amethyst color appears. Add to 5 c.c. of this reagent a few drops of the watery solution obtained above. Observe the lemon-yellow color produced.

If the gastric juice is not extracted with ether before applying this test, certain substances other than lactic acid are present which may give the same reaction. Among these may be mentioned sodium dihydrogen phosphate, alcohol, cane sugar, and glucose.

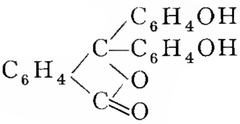
b) *Kelling's test.*—Ten c.c. of stomach contents are diluted about fifteen times with water and treated with one or two drops of a 5 per cent. solution of ferric chloride. A greenish-yellow color shows presence of lactic acid. This test has the same fallacies as Uffelmann's test.

2. *Butyric acid.* — *a*) Treat 2 c.c. of aqueous solution obtained above, by extracting the gastric juice with ether, with a pinch of powdered calcium chloride. Warm slightly. If butyric acid is present, small oil droplets separate out and may be recognized by smell.

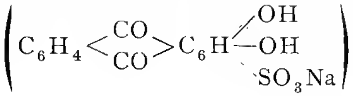
b) Take 5 c.c. of aqueous solution obtained above, evaporate to dryness, and add a few drops of concentrated sulphuric acid and 1 c.c. of alcohol. Notice the odor of ethyl butyrate.

3. *Acetic acid.*—Carefully neutralize above aqueous solution with sodium hydrate. Add to this neutral solution a drop or two of ferric chloride solution. In presence of acetic acid a deep-red color and, on boiling, a precipitate appear. Repeat with acetic acid. Why is it of importance to use a perfectly neutral solution?

V. QUANTITATIVE ESTIMATION OF AVAILABLE HYDROCHLORIC ACID, ORGANIC ACIDS, AND COMBINED ACIDS (TÖPFER).

1. Ten c.c. of filtered gastric contents are titrated (from burette) with a deci-normal solution of sodium hydrate, using phenol-phthalein  as an indicator, until the rose color, which appears on the addition of each drop of the sodium hydrate solution no longer disappears on shaking the receiving flask. The number of cubic centimeters of sodium hydrate solution, employed to bring about this reaction, multiplied by 0.00365 indicates the acidity of the 10 c.c. of gastric juice in terms of hydrochloric acid. To determine the percentage of acidity of the gastric juice, multiply the above figure by 10.

This figure indicates the percentage of the physiologically active (free and combined) hydrochloric acid, as well as that of any acid salts and organic acids, present. Term this result *A*.

2. In a second 10 c.c. of filtered gastric juice, determine the total amount of free acids and acid salts by titrating, as before, with a one-tenth normal solution of sodium hydrate, using as an indicator a few drops of a 1 per cent. aqueous solution of alizarin  $\left(C_6H_4<\begin{matrix}CO\\CO\end{matrix}>C_6H\begin{matrix}-OH\\-OH\\-SO_3Na\end{matrix}\right)$ (alizarin-monosulphonate of sodium). The titration is carried to the point where a pure violet color appears. Calculate acidity. This result is termed *B*. The difference between *A* and *B* gives the amount of combined hydrochloric acid, and is termed *C*.

3. In a third 10 c.c. of filtered gastric juice, determine the free hydrochloric acid by titrating with one-tenth normal sodium hydrate solution, using a few drops of a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol as an indicator. Carry the titration to the point at which the red color, first appearing,

changes to a pure yellow. Calculate acidity. This result is termed *D*. *D* and *C* will thus give the amount of physiologically active hydrochloric acid *E* (viz., the combined and free acid), while the difference between *A* and *E* gives the amount of acid salts and organic acids present.

VI. DETERMINATION OF RATE OF ABSORPTION FROM THE STOMACH.

Take, by mouth, a capsule containing 0.2 g. of potassium iodide. Test saliva, at end of each minute, for iodine by the iodide of starch test learned previously. A positive test should appear within ten to fifteen minutes, if absorption be normal.

VII. DETERMINATION OF MOTOR POWER OF STOMACH.

1. *Fleischer's test.*—Take by mouth, a capsule containing 0.1 g. of iodoform. This is decomposed in the intestine, where iodide of sodium is formed. Test saliva for iodine as above. A positive test should appear in one to one and one-half hours.

2. *Ewald's test.*—Take, by mouth, a capsule containing 1 g. of salol. This is decomposed, on reaching the intestine, into phenol and salicylic acid. Test the urine for salicylic acid by adding a few drops of ferric chloride when a violet coloration appears. A positive test should be observed in one to one and one-half hours.

PANCREATIC DIGESTION.

Owing to the difficulty of obtaining sufficient quantities of true pancreatic juice, it will be necessary to work with the artificial extract of the gland. Such an extract may be made by extracting the gland with tap water containing chloroform. Pancreatic juice exerts a digestive action on proteids, carbohydrates, and fats.

1. *Proteolytic action: Trypsin.*

a) Place about 50 g. of moist fibrin in a flask, add about 200 c.c. of artificial pancreatic juice (200 c.c. of 1 per cent. sodium carbonate solution in which 0.56 g. of Armour's pancreatin are dissolved) and a little chloroform, stopper tightly, and place in the warm closet, at 40° C., for forty-eight to sixty hours. At the end of that time neutralize exactly with sulphuric acid, filter, and divide the filtrate into two equal parts. Part 1: Test for the various albumoses and peptone, as in the peptic digestions. Part 2: Evaporate to one-third its bulk on the water-bath, filtering if a flocculent precipitate appears in the beginning. On cooling, crystals of tyrosin and some leucin generally separate out. Pour the solution from these, wash them in a little cold water, and test for tyrosin. Save the filtrate, and test it for leucin and other products, as in the work on cleavage, under action of concentrated hydrochloric acid.

b) *The rapidity of action of trypsin in different fluids.*—Take five test-tubes, and place in each a small piece of fibrin.

To *A* add 10 c.c. of a 1 per cent. sodium carbonate solution.

To *B* add 10 c.c. of artificial pancreatic juice.

To *C* add 10 c.c. of artificial pancreatic juice neutralized exactly with hydrochloric acid.

To *D* add artificial pancreatic juice slightly acid with hydrochloric acid.

To *E* add 10 c.c. of artificial pancreatic juice and three drops of a 2 per cent. sodium fluoride solution.

Place all in the warm closet, at 40° C., for several hours, noting their condition from time to time. Arrange in your notebook in order of most efficient digestion.

2. Boil artificial pancreatic juice and test its digestive action.

3. *Amylolytic (diastatic action) amylopsin.*—Add a few drops of artificial pancreatic juice to a little starch solution in a test-tube, and after a few minutes test for glucose.

4. *Fat-splitting enzyme, lipase, steapsin.*—Add a cubic centimeter of a freshly prepared water extract of the pancreas to a cubic centimeter of neutral olive oil containing a few drops of blue litmus solution. Place in the closet at 40° C., and report result.

5. To 10 c.c. of a 0.2 per cent. solution of sodium carbonate add a few drops of the oil pancreas mixture used in 4, and shake well. Explain result.

THE BILE.

1. *Reaction.*—Dilute some bile with four parts of water. Immerse a strip of red litmus paper, remove, and wash with water. Note reaction.

2. *Preparation XV: Bile acids.*—These exist, in bile, as the alkali salts. The peculiar fat-dissolving properties of bile are due to the bile salts it contains. Mix 100 c.c. of bile with animal charcoal to form a not too thick paste, avoiding excess, and evaporate on the water-bath to *complete* dryness. Pulverize the residue in a mortar, transfer to a flask, add 25 c.c. of 95 per cent. boiling alcohol and heat on the water-bath for five minutes. Filter. To the cooled filtrate add ether until a permanent precipitate forms. Let the mixture stand for a day in a cool place, and then filter off the crystalline deposit of bile salts. Save the filtrate and prepare from it cholesterin and lecithin by the method already given.

a) Test for bile acids. Dissolve a little of the crystalline precipitate of bile salts in ½ c.c. of water, add a few drops of 10 per cent. solution of cane sugar, cool the mixture under the tap, and then add 1 c.c. of concentrated sulphuric acid. Notice the cherry-red changing to a dark reddish-purple pigment. Avoid a temperature above 70° C. This is called Pettenkofer's reaction, and is not peculiar to bile acids, as it is shown by phenol, fusel-oils, cholesterin, and other substances.

b) The bile salts aid in the emulsification of fat, particularly in the presence of some free fatty acids. Dissolve some of the crystalline bile salts in water. Note the reaction. Add a little olive oil which is slightly rancid and shake. Is the emulsion permanent?

c) Add a few drops of a solution of bile salts to a few cubic centimeters of an albumose solution slightly acidulated with acetic acid. What is the precipitate?

3. *The bile pigments.*

a) *Gmelin's test.*—Pour a cubic centimeter of dog's bile into an evaporating dish, and run into it 1 c.c. of strong nitric acid. Notice the play of colors at the junction of the two fluids. If the bile solution is dilute, proceed as follows: Pour some nitric acid into a test-tube and carefully pour on the top of it the solution to be tested. A greenish-changing-to-red-colored contact-ring indicates bile pigments.

b) *Huppert's test.*—To 10 c.c. of bile add an equal volume of milk of lime, and shake. Filter, wash the precipitate with water, and transfer it to a small beaker containing 25 c.c. of alcohol acidulated with a few drops of sulphuric acid. Warm the beaker and observe the emerald-green color.

c) By oxidation in alkaline solution bilirubin is changed to the green pigment biliverdin. Make a few cubic centimeters of bile slightly alkaline with sodium hydrate and warm, shaking repeatedly in a test-tube. Record the color changes.

For relation of bile pigments to hæmoglobin see "Blood," chap. 4.

4. *Examination of biliary calculi.*—Biliary calculi may be of three kinds: (1) Those consisting of calcium, iron, or copper, in combination with bile pigments (bilirubin-calcium and biliverdin-calcium); (2) cholesterin calculi; (3) calculi of calcium carbonate and phosphate. Examine some of the powdered calculus as follows:

a) Place some of the calculus in a small flask, pour upon it 20 c.c. of ether, and shake thoroughly. Filter through a dry filter, and allow the ether to evaporate from the filtrate. Save the residue on filter paper and in the flask. Test the evaporated ethereal filtrate for cholesterin by tests learned in chap. 1.

b) Remove the solid contents of flask to the filter (previously used) by means of 10 per cent. hydrochloric acid solution. Wash the precipitate with water. The filtrate is saved for future tests for inorganic constituents. Dry the precipitate on filter, and extract several times with 10 c.c. of chloroform. The chloroform should assume a yellow color, due to its solvent action on bilirubin. Filter from insoluble portion. Allow filtrate to evaporate spontaneously. Save the residue (insoluble in chloroform) for later tests, and test the evaporated chloroform filtrate for bile pigments according to tests previously given.

c) Treat the residue (insoluble in chloroform) with a few cubic centimeters of ether. Filter and allow the ether to evaporate. Compare color of residue with that of evaporated chloroform extract. Test this residue for bile pigments as above.

d) Test the acid filtrate obtained in *b*, for inorganic constituents as follows:

(1) *Carbonates.* — Effervescence and liberation of carbon dioxide will be noticed on addition of the hydrochloric acid at beginning of *b*, if carbonates be present.

(2) *Phosphates.*—Evaporate a portion of this acid solution to dryness, take up residue with a few drops of concentrated nitric acid, dilute with 5 c.c. of water, and add 1 c.c. of ammonium molybdate solution. Notice yellow crystalline precipitate.

(3) *Calcium.*—Evaporate the remainder of the acid solution to complete dryness, take up with 5 c.c. of 10 per cent. acetic acid solution, and add 1 c.c. of ammonium oxalate solution. Notice white crystalline precipitate of calcium oxalate.

INTESTINAL JUICE.

Extracts of the small intestine have been shown by Cohnheim to contain a ferment called erepsin, which will split peptone into its ultimate splitting products, but which has no action on the higher proteids. Besides, there has been found by Pawlow and his students a substance called entero-kinase, which will change trypsinogen into trypsin.

Make an extract of dog's pancreas with 1 per cent. sodium carbonate solution, filter, divide into two portions, and to one add some extract of dog's duodenum. Place a piece of fibrin in each solution, and notice speed of digestion. Try also extract of duodenum alone. Give your conclusion.

CHAPTER IV.

THE CHEMISTRY OF THE TISSUES.

THE BLOOD.

The blood is by many considered to be a liquid tissue, connecting all the other tissues of the body, carrying to them, through the medium of the lymph, their nourishment, and receiving from them the waste products.

The blood consists of fibrinogen, serum-globulin, and serum albumin, held in watery solution by the inorganic salts present. In this plasma are suspended the red and white blood corpuscles, blood plaques, and other form elements of the blood.

- Blood
  - Plasma
    - Blood serum
      - Serum albumin
      - Serum-globulin
      - Glucose, calcium salts, phosphates, sodium and potassium chlorides, and other constituents
    - Fibrinogen (yields fibrin)
  - Cellular elements
    - Red corpuscles
      - Lecithin
      - Oxyhæmoglobin
    - White corpuscles
      - Fibrin ferment
    - Blood plaques and other form elements

TESTS FOR THE BLOOD.

1. *Reaction with litmus paper.*—Moisten a piece of litmus paper with a concentrated solution of sodium chloride. Puncture the end of the finger with a sterilized needle, place the drop of blood obtained on the litmus paper for a few seconds, and then wash the blood from the paper by means of the saline solution. As a general rule the alkaline reaction can be clearly made out.

2. Dilute a little blood until a bright-red color is obtained. Add to this (so that a distinct contact layer is formed) a cubic centimeter of a mixture of equal parts of tincture of guaiacum and of ozonized turpentine. Observe the white contact-ring, which later turns to blue.

3. *Hydrogen dioxide.*—Add 1 c.c. of blood to 2 or 3 c.c. of hydrogen dioxide; intense frothing, due to escape of oxygen liberated from the hydrogen dioxide, is observed. The oxyhæmoglobin is gradually decomposed (so-called catalytic action).

4. *Hæmin test* (Teichmann's crystals).—Evaporate a small drop of 6 per cent. sodium chloride solution on a cover-glass. Add a few drops of blood, or, if dried on cloth, a few pieces of the material, powdered or teased as finely as possible. Cover with cover-glass; add enough glacial acetic acid to fill space between the glasses. Continue to add glacial acetic acid to make up for loss by evaporation. When a reddish-brown tinge appears, remove farther from flame, allow acetic acid to evaporate, and examine crystals under microscope. See Plate III.

5. *Spectrum of oxyhæmoglobin.*—Obtain some dilute solution of oxyhæmoglobin and examine with spectroscope. Find absorption bands and refer to absorption bands in sun spectrum (see plate at end of book).

6. *Spectrum of hæmoglobin.*—Reduce the oxyhæmoglobin with ammonium sulphide solution, warming slightly. Now examine spectrum; note change.

7. *Spectrum of methæmoglobin.*—To 10 c.c. of a moderately strong solution of oxyhæmoglobin add one drop of a 10 per cent. solution of potassium ferricyanide (freshly prepared). Notice color of solution, and examine with spectroscope.

8. *Spectrum of carbon monoxide hæmoglobin.*—Pass some gas (water gas) through a solution of oxyhæmoglobin. If gas does not contain carbon monoxide, use pure carbon monoxide (see instructor). Examine through spectroscope. Notice position of absorption bands. Reduce with ammonium sulphide. Observe that the position of the absorption bands does not change as in the reduction of oxyhæmoglobin. This test is of value in cases of suspected poisoning by illuminating gas.

9. Determine the oxyhæmoglobin in your blood by the method of Fleischel (see instructor).

10. *Hæmochromogen* (reduced hæmatin).—To 2 c.c. of blood add 30 c.c. of water and 2 c.c. of concentrated sodium

hydrate solution. Warm gently until color changes to brownish-green. Reduce with ammonium sulphide solution and examine with spectroscope. Determine position of absorption bands and compare with those of oxyhæmoglobin.

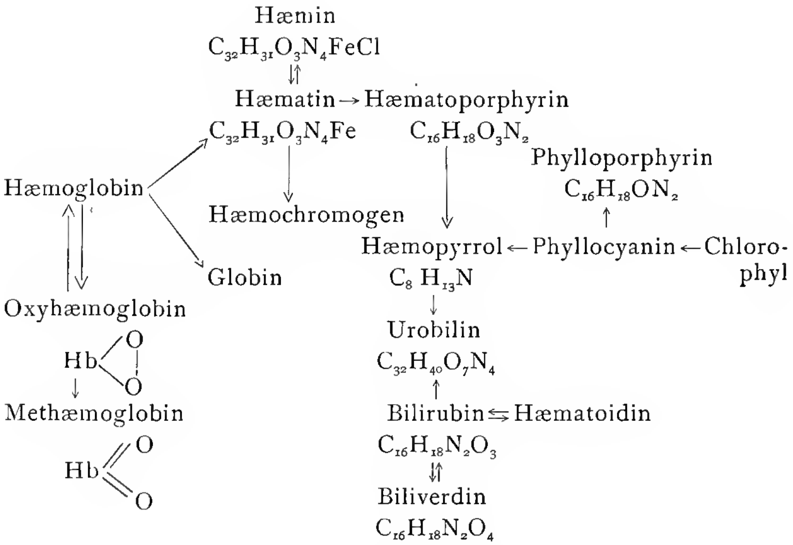
11. *Hæmatoporphyrin* (iron-free hæmatin).—Place in a test-tube a few cubic centimeters of concentrated sulphuric acid. Add a few drops of blood, and notice color. Determine position of absorption bands.

12. Make a blood count by the method of Thoma-Zeiss.

Make drawings of the various spectra in your notebook, and compare them with those given at end of book.

The relation of oxyhæmoglobin to its various derivatives, to bile pigments, and to chlorophyl is best illustrated in the following table:

TABLE SHOWING RELATION OF OXYHAEMOGLOBIN TO ITS DERIVATIVES, TO THE BILE PIGMENTS, AND TO CHLOROPHYL.[1]



---

[1] From Nencki and Marchlenski. *Berichte der deutschen chem. Gesellschaft*, Vol. XXXIV, p. 1687; Nencki and Zaleski, *ibid.*, p. 997.

CLOTTING OF BLOOD.

When blood is shed it coagulates or becomes solid. Three factors are necessary for the process: the presence of fibrinogen (a globulin), the fibrin ferment (not present in the blood until it is shed), and calcium salts. If potassium oxalate is added to the blood, the calcium is precipitated, and coagulation does not take place.

Place in each of four test-tubes about 10 c.c. of horse's blood to which potassium oxalate has been added.Add to tube 1 three or four drops of calcium chloride solution; to tube 2 barium chloride solution. Observe result and record time of clotting. Tube 3 is allowed to stand in ice-box. Record proportion of corpuscles to plasma. For rapid determination some of the blood may be centrifugalized. Tube 4: shake with a little ether and pour some of blood into water. Notice red color. This is called laking. This laking can also be brought about by a number of other substances (solanin, strange sera, lecithin, certain salts, and dilution).

PREPARATION OF BLOOD CONSTITUENTS.

1. *Preparation of serum-globulin and serum-albumin* (see chap. 2).

2. *Preparation XVI: Preparation of fibrinogen.*—Obtain from the instructor 30 c.c. of oxalated plasma (from horse's blood). Add an equal volume of saturated sodium chloride solution. Stir well; remove to porous porcelain plate the fibrinogen which collects on stirring rod. Test the solubility of fibrinogen in water and salt solutions. Test for albumin reactions. Dissolve some of the fibrinogen in dilute sodium chloride solution, add calcium chloride and then a little of the gray layer of corpuscles from tube 3 in clotting experiments. The fibrinogen should clot to a solid mass.

To the solution from which the fibrinogen has been removed add a few drops of acetic acid, and boil. Filter off the coagulated proteid, and test the resulting solution for glucose, albumose, and phosphates.

3. *Preparation XVII: Preparation of oxyhæmoglobin.—a)* Dilute 50 c.c. of defibrinated horse's blood with physiological salt solution; centrifugalize. Decant, add more salt solution, and centrifugalize. Repeat until all the proteids have been removed. Then add 100 c.c. of water and a little ether. Filter cold, then cool the solution to —10° C., add 25 c.c. of alcohol cooled to 0° C., and allow to stand at —10° C. Filter cold, redissolve in water, and reprecipitate with alcohol at —10° C. Spread on porous plate, and dry over sulphuric acid at low temperature.

b) Reichert's Method.—Add to 50 c.c. of defibrinated blood 5 c.c. of ether. Add to this laked blood 10 c.c. of 10 per cent. ammonium oxalate solution. Allow the mixture to stand at room-temperature for half an hour. Notice the abundant formation of crystals. Filter, wash with ammonium oxalate solution, and dry the precipitate on porcelain plate. Examine crystals microscopically and spectroscopically.

THE LIVER.

The liver is of importance on account of its ability to store up carbohydrates as glycogen, to form urea from ammonium compounds, which are derived from the splitting products of proteids, to retain the iron in the body, to elaborate the bile, and to destroy uric acid (in carnivora).

Preparation XVIII: Preparation of glycogen.—The normal liver contains little or no mono- or disaccharides. After death, however, a ferment in the cells converts a considerable part of the liver glycogen into maltose and glucose.

1. Boil 100 g. of the fresh minced liver in 100 c.c. of distilled water, in an evaporating-dish for about one-half hour. Filter. The filtrate should be opalescent. Test the filtrate for proteids; also for glucose by Haines's test. What is the source of this sugar?

Concentrate the filtrate carefully to about 30 c.c. and add 60 c.c. of alcohol. Allow to stand until the heavy precipitate of glycogen separates out; then filter through a small filter, and wash the precipitate with alcohol and dry. Remove the precipitate from the filter and dissolve a little in water. Notice the opalescent solution. Test this solution for a reducing sugar.

Boil a portion of the glycogen solution with iodine solution. What color is obtained? State in your notebooks the physical properties of glycogen.

2. Evaporate the filtrate from the glycogen precipitate obtained above, to dryness on the steam-bath. Extract twice with hot alcohol (10 c.c. each time), and evaporate the alcohol on the steam-bath to dryness. Dissolve the residue in 2 c.c. of a mixture of equal parts of water and of concentrated nitric acid. If present in sufficient amount, crystals of urea nitrate will later separate out.

3. *Iron.*—Grind in a mortar a portion of the liver substance left after extraction of the glycogen, and mix it with a considerable quantity of the fusion mixture. Carefully fuse until the mixture is pure white. Dissolve, after cooling, in a little water, add nitric acid until acid, and boil a moment. Then test for iron with potassium ferrocyanide, and with potassium sulpho-cyanate. What source of error must be avoided in these tests? Where does the liver iron probably come from? State relation to bile pigments.

4. *Preparation XIX: Preparation of nucleo-proteid* (Spitzer's oxydase).—One hundred g. of liver substances are ground in meat-chopper and placed under alcohol over night. Filter, wash with alcohol and ether, and allow to dry. The residue is extracted with 200 c.c. of ½ per cent. sodium carbonate solution, filtered, and the solution carefully neutralized with *n*-10 hydrochloric acid, using phenol phthalein as an indicator. The nucleo-proteid is allowed to settle, washed by decantation, and filtered. When dry, test for phosphorus, iron, and action on peroxide of hydrogen.

THE KIDNEYS.

The kidneys are of importance chiefly from the fact that through them all of the constituents of the urine are excreted. The kidneys contain no substances which are characteristic of them, although Liebermann assumes the presence of a lecithalbumin, which, he claims, has a special importance in the secretion of acid urines, inasmuch as it acts like an acid combining with the alkalies of the salts brought to it by the blood.

Among the various constituents of the kidney only two, cystin and urea, will be considered here.

1. *Preparation of cystin* (Cloetta).—Take 50 g. of beef kidney, grind up with sand, and extract with boiling water for twenty minutes. Filter, add to the filtrate acetate of lead, and filter again. Save the filtrate for urea determination. Suspend this precipitate in water, and pass hydrogen sulphide to remove the excess of lead. Filter, add alcohol to the precipitate, and warm until the precipitate settles out, leaving the solution clear. Decant off the fluid and heat the precipitate (which consists of inosite, taurine, xanthin bases, and cystin) with solution of sodium carbonate, which dissolves the cystin and inosite. Filter and re-precipitate from the filtrate the cystin, by addition of acetic acid. Purify the cystin by dissolving the precipitate in sodium carbonate and re-precipitating with acetic acid.

Test this cystin by tests learned in chap. 2.

2. *Preparation of urea.*—Take filtrate obtained above, pass hydrogen sulphide to remove the excess of lead, filter, evaporate the filtrate to a small volume, and add to the evaporated filtrate an equal volume of strong nitric acid. Evaporate to syrup on water-bath, and proceed as in preparation of urea from urine (chap. 6).

Test by methods given in work on urine.

BONE.

Bone consists of a mixture of organic and inorganic constituents, the relative amounts of these substances present varying in different bones and at different ages. The organic part of bone contains a large amount of a collagen, called ossein, from which gelatin may be derived by hydration (see chap. 2). The inorganic part consists chiefly of phosphates and carbonates of calcium and magnesium, along with fluorides of sodium and potassium.

With the hydrochloric acid solution obtained previously (chap. 2) in the preparation of gelatin, test for inorganic constituents as follows:

1. Evaporate the solution to dryness on the water-bath. Take up the residue with 10 per cent. nitric acid. Add to this solution an excess of ammonium molybdate solution. Warm slightly,

and observe the yellow crystalline precipitate of ammonium phosphomolybdate. This shows presence of phosphoric acid.

2. Filter and alkalinize the filtrate with ammonium hydrate. Add a little ammonium chloride solution (to prevent precipitation of the magnesium present), and then add ammonium oxalate solution. Notice the white crystalline precipitate of calcium oxalate which comes down in a short time.

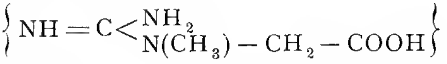
3. Filter from the calcium oxalate and add to the filtrate a few cubic centimeters of disodium hydrogen phosphate solution. A white crystalline precipitate of ammonium magnesium phosphate settles out on standing.

THE MUSCLE.

From a chemical standpoint, muscular tissue is subject to very great changes in composition. The isolation of the different morphological elements has not as yet been completed, owing to the complexity of the problems involved. However, a few of the constituents of this tissue will be studied. The proteids, which form the chief part of the solids of the muscle, are perhaps the most important constituents, inasmuch as the metabolic processes are largely dependent upon their inter-relation with the glycogen. Other constituents, such as kreatin, xanthin bases, lactic acid, and glycogen, are present in varying amounts, and will be studied in more or less detail.

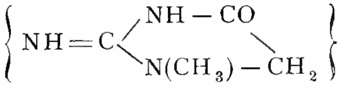
1. *Preparation of syntonin* (see chap. 2).
2. *Preparation XX:*

Preparation of kreatin



$$\left\{ NH = C \begin{matrix} \diagup NH_2 \\ \diagdown N(CH_3) - CH_2 - COOH \end{matrix} \right\}$$

and kreatinin



—Mix 500 g. of finely chopped beef, as free as possible from fat and tendon, with 500 c.c. of water. Heat on water-bath at 50--60° C. for one-half to three-fourths of an hour. Filter through muslin. To the filtrate add a few drops of acetic acid, and boil for a short time, in order to coagulate the proteids. Filter through muslin, and add to the cooled filtrate basic lead acetate as long as a precipitate forms (25 c.c. generally suffices). This latter treatment is for the purpose of removing the phosphates, chlorides, and sulphates which may interfere with the later work. Filter through a folded filter and free the filtrate from lead by means of hydrogen sulphide. Filter off the lead sulphide and evaporate the colorless filtrate on water-bath to a thin syrup. Let this syrup stand for twenty-four to forty-eight hours in a cool place to crystallize. Crystals of kreatin will separate out. Separate the crystals from the mother-liquor and dry them on a porcelain plate. If the crystals are highly colored, redissolve them in water, and decolorize the solution with animal charcoal. Hand in a sample of this kreatin and proceed with the remainder as follows: Test its solubility in water (hot and cold), alcohol, and ether. Test its reducing action on Fehling's or Haines's solution. Note here that no separation of cuprous oxide occurs, although reduction has taken place. As kreatin is best identified by converting it into kreatinin, which gives striking reactions, treat the large part of your kreatin crystals as follows: Place the kreatin crystals in a small flask, dehydrate by pouring upon them 10 c.c. of dilute sulphuric acid, and heat for one-half hour on water-bath, adding water to replace that lost by evaporation. Dilute the contents of the flask with 25 c.c. of water, and while solution is still warm add sufficient barium carbonate to neutralize the excess of acid. When neutral, filter and evaporate the filtrate over water-bath to small bulk (5 c.c.). With this solution perform the following tests:

a) *Kreatinin zinc chloride* ($((C_4H_7N_3O)_2ZnCl_2)$).—Place a few drops of the kreatinin solution upon a watch-glass and add to it a few drops of alcoholic zinc chloride solution. Note formation of crystals. Examine microscopically, and observe the clumps or rosettes of prisms or fine needles.

b) *Weyl's reaction.*—To a portion of the kreatinin solution add, drop by drop, a dilute solution of sodium-nitro-prusside ($Na_2NOFe(CN)_5$) until the solution is colored yellow; add to this yellow solution, drop by drop, sodium hydrate solution. A fine, transient, ruby-red color appears, which quickly fades into

a straw-yellow. Strongly acidify this latter yellow solution with acetic acid, warm, and note the greenish-blue coloration. This last color is due to formation of Prussian blue. If ammonium hydrate solution be used instead of sodium hydrate solution, this reaction does not appear with kreatinin. It should be noted here that acetone, which may appear, particularly in the urine, along with kreatinin, gives a reaction which is somewhat similar to the above, but which is distinguished from the kreatinin reaction by the reddish-purple color given by the former on acidifying with acetic acid.

c) *Jaffe's reaction.*—To a portion of the kreatinin solution add an aqueous solution of picric acid and then a few drops of sodium hydrate solution. Note the deep-red color produced.

3. *Proteids in muscle.*—Mix 100 g. of finely chopped lean beef with 300 c.c. of water and allow to stand two hours. Filter first through muslin and then through filter paper. Save the residue for future extraction of myosin and proceed with the filtrate as follows:

a) Test the reaction of the filtrate toward delicate litmus paper and also toward a lacmoid solution. Note that the filtrate reacts acid toward litmus and alkaline toward lacmoid solution (it may, however, be acid toward lacmoid, if the meat has lain for some time before use). The presence of acid monopotassium phosphate (which reacts more sensitively with litmus) and of alkaline dipotassium phosphate (to which lacmoid is more sensitive) explains this peculiar result.

b) Take 50 c.c. of filtrate, place in beaker, and heat gradually on water-bath. Place a thermometer in the solution, and notice that a coagulation takes place as the temperature approaches 50°. Heat solution to 56° C. and filter. This coagulum appearing between 50° and 56° is paramyosinogen. Place the filtrate from the paramyosinogen on water-bath and allow it to warm to 65° C. Note that a second coagulation takes place. This second coagulum is myoglobulin. Filter and heat the filtrate to 75° C., when a third coagulum (myo-albumin) appears.

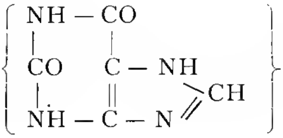
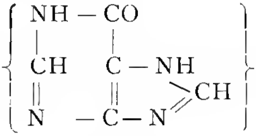
c) *Extraction of myosin.*—Extract the meat residue obtained at beginning of 3 with a 15 per cent. ammonium chloride solution, adding enough of the latter to form a moderately thick mixture. Cover the vessel with a glass plate, allow to stand twenty-four hours, and filter. Test the filtrate, which contains the myosin, as follows:

(1) Pour a few drops of the filtrate into a relatively large bulk of distilled water. Notice precipitate of myosin.

(2) Place a few drops of the filtrate upon a piece of rock-salt. As the solution becomes saturated with sodium chloride, a film of myosin appears.

(3) Boil a few cubic centimeters of the filtrate; the myosin is coagulated. Filter and test filtrate for calcium.

4. *Preparations XXI and XXII:*

Preparation of xanthin  *and hypoxanthin*  .—Dissolve 50 g. of Armour's or Liebig's extract of beef in 500 c.c. of water. Add to the solution basic lead acetate as long as a precipitate forms. Filter through a folded filter, and pass hydrogen sulphide through filtrate to remove excess of lead. Filter and evaporate the clear filtrate to three-fourths its bulk, to remove hydrogen sulphide. After cooling, alkalinize with concentrated ammonium hydrate solution, filter if necessary, and add to filtrate 100 c.c. of a 2½ per cent. silver nitrate solution. Collect the precipitated xanthin and hypoxanthin silver compounds on a smooth filter, and wash with water. Save the filtrate obtained here for the preparation of sarco-lactic acid. With the precipitate proceed as follows: Place the precipitate in a flask, and pour upon it a mixture of 100 c.c. concentrated nitric acid and 100 c.c. of water. Add 1 g. of urea (to prevent formation of nitrogen trioxide, which tends to

destroy the bases) and heat to boiling. Allow to cool for twenty-four hours. On cooling, the hypoxanthin silver nitrate crystallizes out, while the xanthin silver nitrate remains in solution. Filter. Save filtrate for preparation of xanthin, and proceed with precipitate as follows:

a) Wash the precipitate into a small Erlenmeyer flask with water. Add 1 c.c. of dilute hydrochloric acid, warm to 30° C., cork the flask, and shake for a little time. This treatment decomposes the hypoxanthin silver nitrate, giving silver chloride and hypoxanthin nitrate. Too strong heating is to be avoided, as the mixture of nitric and hydrochloric acid will destroy the hypoxanthin under these conditions. Filter off the silver chloride, alkalinize the filtrate with ammonium hydroxide, and evaporate on water-bath to dryness. This residue contains hypoxanthin, ammonium nitrate, and ammonium chloride. Treat this residue with a few cubic centimeters of water, which dissolves the two ammonium salts and leaves the hypoxanthin as a residue. Filter, wash with water, and test the hypoxanthin as follows:

(1) Test its solubility in water (hot and cold), mineral acids, and ammonia.

(2) Place a few crystals of hypoxanthin in a porcelain dish, add a few cubic centimeters of fuming nitric acid, and evaporate over water-bath to dryness. Notice the yellowish residue (if ordinary colorless nitric acid be used, the residue is practically colorless). Moisten this yellowish residue with sodium hydrate solution, and observe the orange-yellow coloration.

b) Neutralize the filtrate obtained on filtering out the hypoxanthin silver nitrate with concentrated ammonium hydroxide. Note the brown-red precipitate or reddish solution, which upon standing deposits reddish flakes. Filter, wash with water, suspend the precipitate in water, add a few drops of ammonium hydroxide, warm, and add a few drops of ammonium sulphide. Shake thoroughly, filter, and evaporate the filtrate over water-bath to dryness. This gives the xanthin, although not in a very pure condition.

(1) Test solubility of xanthin in water, alcohol, ether, alkalies, and acids.

(2) Place some of the xanthin in an evaporating dish, add a few cubic centimeters of nitric acid, and evaporate on water-bath to dryness. Note yellow residue, which on addition of caustic soda becomes deep-red.

(3) *Weidel's reaction.*—Place a portion of the xanthin in a porcelain dish and dissolve by warming with a few drops of bromine water. Evaporate to dryness, and permit fumes of ammonium hydroxide to come in contact with this residue. Note red coloration.

5. *Preparation XXIII: Preparation of sarco- or para-lactic acid* (CH_3-CHOH-COOH).—Take the filtrate from the xanthin and hypoxanthin silver salts and evaporate on water-bath to remove the ammonia. Pass hydrogen sulphide through the evaporated solution to remove the silver. Filter and evaporate to consistency of a syrup. Extract the residue with 100 c.c. of 95 per cent. alcohol, filter, and evaporate the filtrate over water-bath. Dissolve residue in 75 c.c. of water, add 25 c.c. concentrated sulphuric acid, and extract the solution in a separatory funnel, three or four times, with an equal volume of ether. Filter these ethereal extracts into a dry flask. Evaporate these extracts to dryness. Test the residue for lactic acid as follows:

a) *Uffelmann's test.* — See "Lactic Acid" under "Gastric Juice."

b) Dissolve a portion of residue in water and boil with an excess of freshly precipitated zinc carbonate. Filter, evaporate filtrate until crystallization commences, add a little alcohol, and allow to stand twenty-four hours. Filter off the crystallized zinc-sarco-lactate and recrystallize it from water.

THE BRAIN.

The chemical examination of the brain is of especial interest on account of the selective action which many drugs have on the nervous system. The taking up of anæsthetics by the central nervous system has been explained by the very large amount of lecithans and cholesterin present.

Preparation XXIV: Preparation of cholesterin.—Take one sheep's brain and carefully remove all the larger blood-vessels

and any parts of the dura mater that may have been left. Grind fine in a meat chopper and add 50 c.c. of acetone. Heat in a stoppered flask almost to boiling for two hours (avoiding a free flame). Cool and filter. The filtrate contains some of the extractives, which may be disregarded. The residue is dried in warm closet and then put into a 200-c.c. flask with 60 c.c. of carbon disulphide over night. Filter, evaporate the filtrate to dryness, dissolve the residue in 20 c.c. of ether, filter, and add 50 c.c. of acetone to the filtrate. The precipitate consists of the various lecithans (lecithin, myelin, cephalin). Notice the relatively large amount of lecithans as compared with those from yeast. The ether acetone filtrate is evaporated and leaves behind the cholesterin, which can be purified by recrystallization from alcohol. This is a very convenient method for preparing large quantities of cholesterin.

Preparation XXV: Preparation of cerebrin.—Take one sheep's brain, extract twice with 100 c.c. of hot alcohol. Filter hot. Unite the filtrates and cool to 0° C. Filter and recrystallize the precipitate twice by heating with 20 c.c. of glacial acetic acid and allowing the cerebrin to settle out from the cooled solution, wash with alcohol and ether, and dry over sulphuric acid. Cerebrin is a constituent of the medullary sheath of the nerve fiber.

THE PANCREAS.

The pancreas is of two-fold importance: (1) it secretes a digestive juice which contains ferments capable of digesting all general classes of foods: (2) it is of great value in the metabolic processes of the system, as it aids in the transformation of glucose in the body.

Chemically considered, the pancreas contains the ordinary elements found in other tissues, and, besides, the zymogens, which give rise to the important ferments found in the secretion of the gland. One of these ferments will be isolated and studied.

Preparation XXVI: Preparation of trypsin (Heidenhain's method).—Grind up 50 g. of finely divided pancreas with sand. Mix this mass with 1 per cent. acetic acid solution (using 1 c.c. of acid for every gram of gland). Add to this mixture ten parts of glycerin for every gram of gland used. Allow to stand for three days, and filter. Precipitate the trypsin from the glycerin solution by use of alcohol (95 per cent.). Remove fat by shaking out this precipitate with ether. Dry the purified precipitate on a porcelain plate, and test its digestive action by dissolving in 1 per cent. sodium carbonate solution and adding some fibrin. Observe time of digestion and products of this digestion.

THE STOMACH.

The pepsin necessary for the gastric digestion is secreted from certain cells situated in the wall of the stomach. The purest pepsin so far prepared was made by Pekelharing from the stomach wall.

Preparation XXVII: Preparation of pepsin.—Take a solution of Armour's pepsin or the liquid formed by permitting the stomachs of several pigs to undergo digestion in acid solution, and dialyze against flowing water until the acidity has decreased to 0.022 per cent. The precipitated pepsin is centrifugalized and the supernatant liquid decanted. This liquid still retains some pepsin. The precipitated pepsin is again dissolved in a small quantity of 0.022 per cent. hydrochloric acid, dialyzed, centrifugalized, and collected on a smooth filter paper. The water is pressed out from the precipitate, and the precipitate dried over sulphuric acid in vacuum.

CHAPTER V.

THE MILK.

THE CHEMICAL AND SANITARY EXAMINATION OF MILK.

In the introduction to chap. 2 the principal functions of the three classes of foods have been briefly outlined. The proportion in which the fats, carbohydrates, and proteids should be present in the daily diet, in order that they may best perform their function, varies largely. It depends on climate, and on the health and occupation of the individual. As most of our ordinary articles of diet (commonly called foods) contain all of the above three classes of foods, the determination of the fats, carbohydrates, proteids, and the inorganic salts (ash) is a matter of some importance. Milk, the only food of the infant, and even in adult life an important article of diet, may serve as an example of the others.

I. CHEMICAL EXAMINATION OF MILK.

1. *Determination of fat.*—This is determined by the Babcock method, the principle of which consists in the destruction of all the organic matter except the fat by sulphuric acid. The flask in which the reaction is carried on is so arranged that the percentage of fat can be read directly on the neck of the flask. Determine the percentage of fat in the sample of milk submitted to you. Consult instructor about use of the Babcock apparatus.

For foods other than milk the Soxhlet method of ether extraction is used for the determination of the fat. Consult text-books on food analysis.

2. *Total solids.*

a) *By specific gravity method* (for quick results).—Determine the specific gravity of the sample of milk, and by use of the accompanying table find its total solids, taking into account the percentage of fat found in previous experiment.

TABLE FOR THE CALCULATION OF TOTAL SOLIDS FROM FAT AND SPECIFIC GRAVITY (RICHMOND'S FORMULA).

Specific Gravity.	Fat.							
	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2
1.0300	10.65	10.75	10.90	11.00	11.15	11.25	11.35	11.50
1.0305	10.75	10.90	11.00	11.15	11.25	11.35	11.50	11.60
1.0310	10.90	11.00	11.15	11.25	11.40	11.50	11.60	11.75
1.0315	11.00	11.15	11.25	11.40	11.50	11.60	11.75	11.85
1.0320	11.15	11.25	11.40	11.50	11.60	11.75	11.85	12.00
1.0325	11.25	11.40	11.50	11.60	11.75	11.85	12.00	12.10
1.0330	11.40	11.50	11.65	11.75	11.85	12.00	12.10	12.25
1.0335	11.50	11.65	11.75	11.85	12.00	12.10	12.25	12.35
1.0340	11.65	11.75	11.85	12.00	12.10	12.25	12.35	12.45
1.0345	11.75	11.90	12.00	12.10	12.25	12.35	12.50	12.60

Specific Gravity.	Fat.						
	3.3	3.4	3.5	3.6	3.7	3.8	3.9
1.0300	11.60	11.75	11.85	11.95	12.10	12.20	12.35
1.0305	11.75	11.85	11.95	12.10	12.20	12.35	12.45
1.0310	11.85	12.00	12.10	12.20	12.35	12.45	12.60
1.0315	12.00	12.10	12.20	12.35	12.45	12.60	12.70
1.0320	12.10	12.20	12.35	12.45	12.60	12.70	12.80
1.0325	12.20	12.35	12.45	12.60	12.70	12.80	12.95
1.0330	12.35	12.45	12.60	12.70	12.85	12.95	13.05
1.0335	12.45	12.60	12.70	12.85	12.95	13.05	13.20
1.0340	12.60	12.70	12.85	12.95	13.05	13.20	13.30
1.0345	12.70	12.85	12.95	13.10	13.20	13.30	13.45

b) *By gravimetric method* (for more accurate result).—Weigh 5 g. of milk into a weighed platinum dish. Evaporate on water-bath, and dry to constant weight in drying oven at 105° C. This gives percentage of total solids.

3. *Determination of ash* (inorganic salts).—The residue from the previous determination, after weighing, is carefully heated until all the organic material has become charred without catching fire. The mass is then heated to a dull redness until the ash is perfectly white. Cool, weigh, and calculate percentage of ash. This method does not give a good indication of the amount of inorganic salts present and is of value only in comparative work.

4. *Determination of proteids.*—Ten c.c. of milk are placed in a 250-c.c. beaker and diluted with 100 c.c. of water (for human milk 60 c.c. of water is used). The mixture is then heated nearly to boiling, 2 c.c. of saturated alum solution is added, and when

the fluid begins to boil 5 c.c. of Stutzer's[1] reagent is run in and the mixture kept at the boiling-point for five minutes. The resulting precipitate, which settles quickly, is washed with hot water until the filtrate shows no reaction for sulphates. Save the filtrate and washings for 5. The filter papers and contents are then placed in a Kjeldahl flask and treated as directed in chap. 6 for the determination of nitrogen in the urine. For the determination of total nitrogen in milk proceed as directed in urine. The amount of nitrogen multiplied by 6.25 gives the amount of proteid.

5. *Determination of lactose.*—The filtrate and washings from 4, which are now free from proteids, are transferred to a graduated 200-c.c. flask, and an aliquot part titrated against Fehling's or Purdy's solution, as directed in chap. 6 for the determination of glucose in the urine. Base the calculation for lactose on the fact that the amount of filtrate required to reduce 20 c.c. of Fehling's solution is equivalent to 0.134 g. of lactose. (For glucose 0.1 g. is equivalent to 20 c.c. of Fehling's solution.)

From a large number of samples the composition of normal milk has been found to be about as follows (König):

	Cow.	Human.	Malted Milk.
Water	87.17	87.41	4.3
Total solids	12.83	12.59	
Fat	3.69	3.78	1.3
Lactose	4.88	6.21	79.6 (maltose) 3 insoluble carbohydrate
Proteids	3.55	2.29	2.5
Ash	0.71	0.31	10.0

---

[1] *Stutzer's reagent.*—One hundred g. of crystallized, chemically pure copper sulphate is redissolved in five liters of water, and treated with 2½ g. of glycerin. Dilute sodium hydrate is then added, which precipitates the copper as cupric hydroxide. The precipitate is then filtered off and washed with water containing 0.5 per cent. glycerin by decantation until the filtrate is free of alkali. The filtrate must be entirely colorless, indicating the absence of copper. If it be still blue, more sodium hydrate must be added. The filtered mass is then treated with water containing 10 per cent. of glycerin and so diluted that it may be readily drawn up into a pipette. The mixture should be kept in a tightly stoppered blue or brown bottle. The proportion of copper hydroxide per cubic centimeter may be determined by drying a measured volume of the material and weighing the residue. Before using this reagent it should be shaken vigorously, in order to mix the precipitate uniformly.

A comparison of human milk with cow's milk shows a difference in the proportion of carbohydrates to proteids. Cow's milk must therefore be modified in order to be useful for the feeding of young children. The most common method is to dilute the milk to decrease the proteids, and to add lactose to make up for the lack of carbohydrates. To obviate the dilution of the fat, Dr. Backhaus has suggested the addition of very rich cream.

For the examination and analysis of other foods besides milk consult Hammarsten and special text-books.

II. SANITARY EXAMINATION OF MILK.

Milk is often adulterated with preservative agents, and hence these should always be tested for in cases in which the milk is found to have an abnormal reaction.

1. *Reaction.*—Moisten a piece of blue litmus paper and test milk for acidity. Milk should be neutral in reaction.

2. *Sodium carbonate.*—To hide the acid reaction of a spoiled sample of milk, sodium carbonate is often added. Test as follows: Ten c.c. of milk are mixed with 10 c.c. of alcohol (96 per cent.) and a drop of rosolic acid solution (1 : 100). Pure, unadulterated milk produces a brownish-yellow color, but in the presence of sodium carbonate or sodium bicarbonate a rose color is obtained. For greater precision in doubtful cases the questionable sample should be compared with known unadulterated milk. Phenol phthalein solution may not be used as an indicator in place of rosolic acid in this test. By this method 0.05 per cent. of these carbonates may easily be detected.

3. *Detection of salicylic acid.*—Acidify 20 c.c. of milk with two or three drops of sulphuric acid, and shake with about an equal amount of ether. The greatest possible part of the ether solution is drawn off and evaporated, the residue extracted with 40 per cent. alcohol and filtered, and 5 c.c. of the filtrate treated with a few drops of ferric chloride. A violet color shows the presence of salicylic acid and some other oxy acids of benzol.

4. *Detection of borax and boric acid.*—For qualitative tests about 100 c.c. of milk are made alkaline with milk of lime, evapo-

rated to dryness, and incinerated. The resulting carbon need not be completely burned. The heating is stopped as soon as intumescence ceases. The ash is dissolved in the least possible amount of hydrochloric acid, filtered off from the carbon, evaporated to dryness, and the excess of hydrochloric acid completely driven off. The resulting white crystalline residue is treated with a few drops of a tincture of turmeric and very dilute hydrochloric acid, and dried on a water-bath. The presence of the slightest trace of boric acid gives to the dry residue a beautiful vermilion or cherry-red color (Meissel). By the described method it is possible to detect with certainty 0.001 to 0.0005 g. of boric acid in the ash or 0.001 to 0.002 per cent. in the milk.

To render the milk alkaline, lime-water is preferable to the caustic alkalies because, in fusing, the latter are liable to cover over particles of unburned organic matter and make the conversion to ash difficult. Only very dilute hydrochloric acid must be used in testing for boric acid, since the concentrated acid itself gives with turmeric tincture a red color. The coloration produced by boric acid is distinguished from that produced by hydrochloric acid by the fact that it does not disappear by treatment with water in the cold, but only after long boiling. The color caused by hydrochloric acid disappears as soon as it is diluted with water. The acid is easily soluble in alcohol, and a trace of it in alcoholic solution colors the flame of the Bunsen burner an intense green. With alcohol, boric acid ester is formed. If the alcohol be ignited, a flame tinged with green is produced.

5. *Detection of formaldehyde.*—Place 10 c.c. of milk in a test-tube; carefully pour down the side of the tube so that it collects at the bottom 3 c.c. of concentrated hydrochloric acid. On standing, a pink color is formed at the juncture of the two liquids. This is characteristic of formaldehyde in presence of casein.

The above are the simplest methods for detecting the more common adulterants. For quantitative methods and more detailed information consult special text-books.

CHAPTER VI.

THE CHEMISTRY OF THE EXCRETIONS.

THE URINE.

The constituents of normal urine are derived in part from the metabolic products of the tissues, and in part from the products formed or taken into the alimentary canal. The variations in nutrition (tissue changes) and waste are easily recognized by a careful study of the urine. Although all forms of disease cannot be diagnosed by an examination of the urine, yet no marked disorder can be present without some change manifesting itself in the urine.

The normal human urine is a watery solution of different inorganic and organic basic and acid bodies. The chief representatives of these are, on the one hand, potassium, sodium, ammonium, calcium and magnesium salts of hydrochloric, carbonic, phosphoric, and sulphuric acids (ordinary or ethereally bound to aromatic alcohols); on the other hand, we have urea, kreatinin, xanthin bases, uric acid, oxalic acid, hippuric acid, and other fatty and aromatic acids, to which coloring matters and slight amounts of proteid bodies (such as mucin) are bound. Besides these constituents the gases, oxygen, nitrogen, and carbon dioxide exist in solution (Neubauer and Vogel). The average composition of the urine may be seen from the following table, based upon parts of substance per thousand:

Solids:		
Organic:		
Urea	26.00	
Kreatinin	1.50	
Urates	1.75	
Mucin and coloring matter	.25	
Uric acid traces		29.50

Inorganic:		29.50
Sodium, potassium, and calcium phosphates	6.25	
Sodium and potassium chlorides	9.25	
Sodium and potassium sulphates	5.75	
		21.25
Water		949.25
		1,000.00

I. PHYSICAL PROPERTIES OF NORMAL URINE.

Note general appearance, color, transparency, odor, specific gravity, consistence, and reaction of your urine. Determine whether the reaction, if alkaline, be due to fixed or ammoniacal alkalies.

The urine is subject to more or less rapid changes after it is voided. To show this allow some urine to stand in an open test-tube for a day or two, and then note the points mentioned above, paying attention particularly to the reaction, odor, and transparency. Explain the changes observed. Look up in text-book the variations which may occur in these general characteristics of the urine, and explain the cause of each variation.

II. DETECTION AND ESTIMATION OF INORGANIC CONSTITUENTS OF NORMAL URINE.

1. *Chlorides.*—The chlorides of the urine consist chiefly of sodium chloride, with a small amount of potassium and ammonium chlorides.

a) *Qualitative determination.*—To a few cubic centimeters of urine add a drop or two of concentrated nitric acid and then a cubic centimeter of silver nitrate solution. Notice precipitate formed. What is this? Add to the cloudy solution some ammonium hydrate. What occurs? Explain result.

b) *Quantitative estimation of the chlorides* (*Volhard's method*).—Exactly 10 c.c. of the urine (proteid, if present, must be removed) are placed in a 100-c.c. graduated flask, and 5 c.c. of concentrated nitric acid (chlorine-free) are added. Add about 50 c.c. of water, allow exactly 20 c.c. of standard silver nitrate solution (containing 29.075 g. of silver nitrate per liter; hence each cubic centimeter corresponding to 0.1 g. of sodium chloride) to flow in. Shake flask well (avoiding loss of fluid) and fill flask to mark with distilled water. Carefully shake flask and filter contents through a dry filter. Measure off accurately 50 c.c. of the filtrate, add 3 c.c. of saturated ferric sulphate solution, and allow potassium sulpho-cyanate solution (8.3 g. of potassium sulpho-cyanate per liter, each cubic centimeter corresponding to ½ c.c. of the silver nitrate solution) to flow in from burette until the liquid above the precipitate has a permanent red color. The calculation is as follows: Subtract from the 20 c.c. of silver nitrate solution originally used the number of cubic centimeters of potassium sulpho-cyanate solution used to bring about the permanent red color in the 50 c.c. of filtrate. Multiply this figure by 2 to obtain quantity necessary for the 100 c.c. As the sulpho-cyanate solution is of such a strength that 1 c.c. corresponds to ½ c.c. of silver nitrate, divide this figure by 2 to obtain number of cubic centimeters of silver nitrate solution used in precipitating the chlorides present in the 10 c.c. of urine (diluted to 100). Each cubic centimeter of the silver nitrate solution neutralizes 0.01 g. of sodium chloride; hence multiply the figure obtained, after dividing by 2, by 0.01. This gives the amount of sodium chloride in 10 c.c. of urine. Multiply by 10 to obtain percentage composition.

2. *Phosphates.*—In the urine phosphoric acid occurs in combination with the alkaline earths, and in part with the alkalies.

a) *Qualitative determination.*—Add to the urine some sodium hydrate solution. Notice precipitate formed. What is this precipitate? What is the importance of this reaction? Filter off this precipitate, acidify a few cubic centimeters of the filtrate with a few drops of acetic acid, and add a little ferric chloride solution. Notice yellowish-white precipitate of ferric phosphate. This latter precipitate comes from the decomposition between the alkaline phosphates and ferric chloride, while the former precipitate shows the presence of the earthy phosphates.

b) *Quantitative determination.*—When a solution of di-sodium phosphate, acidified with acetic acid, is treated with a solution of uranium nitrate, a dirty-looking, white precipitate of uranium phosphate is thrown down. If potassium ferrocyanide be present, a brownish color appears just at point of neutralization of all the dibasic phosphates by the uranium solution.

$$2NaH_2PO_4 + UO_2(NO_3)_2 = UO_2(H_2PO_4)_2 + 2NaNO_3\ .$$

Apply this determination as follows: 50 c.c. of clear filtered urine are treated with 5 c.c. of acetic acid mixture (10 g. of sodium acetate and 10 g. of glacial acetic acid in 100 c.c. of water), the object being to transform any monacid sodium phosphate into the diacid sodium phosphate. Add a few drops of potassium ferrocyanide solution, and heat the mixture to boiling. Titrate this boiling solution from burette with standard uranium nitrate solution (44.78 g. uranium nitrate in 1 liter of water, giving a solution of such strength that 20 c.c. corresponds to 0.1 g. P_2O_5) until a permanent brownish color is noticed in the precipitate. (This can best be determined by testing separate portions on porcelain plate with the potassium ferrocyanide solution.) Calculate the percentage of P_2O_5 in the urine.

3. *Sulphates.*—Sulphuric acid occurs in the urine, partly in combination with sodium and potassium, and partly with aromatic bodies of putrefactive origin, such as indoxyl and skatoxyl. The first are the ordinary neutral sulphates, while the latter are the conjugate or ethereal sulphates.

a) *Qualitative determination.*—To 25 c.c. of urine add barium chloride solution until no further precipitation occurs. What is this precipitate? Filter and add to the filtrate 2 c.c. of concentrated hydrochloric acid and boil. Now add to this acidified solution a few drops of barium chloride solution. Notice further precipitation. Explain results.

b) *Quantitative determination.*

(1) *Total sulphates.*—One hundred c.c. of filtered urine are treated with 5 c.c. of concentrated hydrochloric acid and boiled for fifteen minutes. While boiling, precipitate by addition of 2 c.c. of a saturated barium chloride solution. Warm until the barium sulphate has completely settled. Filter through a small ash-free filter (removing the precipitate quantitatively to the filter). Wash the precipitate on filter with water until the washings show no trace of chlorides. Dry at 100° C. Transfer the filter and contents to a weighed and ignited crucible and ignite contents until residue is white. Calculate amount of sulphuric acid in the urine.

(2) *Ethereal sulphates* (*Salkowski's method*).—Treat 200 c.c. of filtered urine with an equal volume of a barium solution

(2 vols. of saturated barium hydrate solution and 1 vol. of saturated barium chloride solution). Filter through a dry filter and measure off exactly 100 c.c. of filtrate, which consists of ethereal sulphuric acids in solution. Treat this 100 c.c. with 10 c.c. of concentrated hydrochloric acid, boil for fifteen minutes, and warm on water-bath until the precipitate has completely settled out. Filter and proceed with the precipitate as directed in estimation of total sulphates. The difference between total sulphates and ethereal sulphates gives the ordinary mineral sulphates. Besides these there are present in the urine certain sulphur compounds, such as cystin, in which the sulphur is in unoxidized form.

4. *Carbonates.*—The carbonates present are, for the most part, those of sodium and potassium.

Qualitative determination. — Evaporate a few cubic centimeters of the urine to dryness on water-bath. Add a few drops of 10 per cent. hydrochloric acid solution. Observe effervescence. Notice effect of this gas when passed into lime-water.

III. DETERMINATION OF TOTAL SOLIDS IN THE URINE.

1. Take 20 c.c. of urine and evaporate it, carefully, over water-bath, in a previously weighed porcelain dish. Continue the heating in a drying oven until the weight of dish and contents remains constant at 105° C. Calculate total solids.

2. Determine the specific gravity of the urine and multiply the last two figures of this specific gravity by 2.33 (Häser's coefficient). This gives approximately the number of grams of solids in each 1,000 c.c. of urine.

Instead of Häser's coefficient, Haines's coefficient may be used. This is 1.1. The result gives the number of grains of solids in each fluid ounce of urine.

IV. DETERMINATION OF AMMONIA.

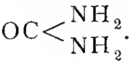
Folin's method. — Twenty-five c.c. of urine are accurately measured and placed in a flask. Add 8--10 g. of sodium chloride, 5--10 c.c. of petroleum or toluol (to prevent foaming), and 1 g. of dry sodium carbonate. Keep flask at 20--25° C. and draw a stream of air through (using 600--700 L. of air in one to one and one-half hours) the flask until all of the ammonia liberated is drawn out. Allow this ammonia containing air to pass through

a glass wool plug (to hold back other alkalies in suspension) and then through a flask containing a measured amount of *n*-10 sulphuric acid. Titrate back the excess of acid with *n*-10 sodium hydrate, using alizarin-red as an indicator, until a red color, not a violet color, is obtained. Calculate the amount of ammonia given off in the process.

V. DETERMINATION OF TOTAL NITROGEN.

Kjeldahl's method.—The principle of the method is the following: The nitrogenous bodies are decomposed by means of strong sulphuric acid, the carbon being oxidized to carbon dioxide, the hydrogen to water, and the nitrogen being converted into ammonia, which combines with the sulphate radical, forming ammonium sulphate. On the addition of alkali (sodium hydrate) the ammonium sulphate is decomposed, giving ammonia, which is distilled into a known amount of *n*-10 sulphuric acid. Proceed as follows: Place 10 c.c. of urine in a clean 300-c.c. Kjeldahl flask, add 10 c.c. of concentrated sulphuric acid and a few crystals of potassium permanganate (or ½ g. of yellow mercuric oxide). Heat the inclined flask under the hood until the contents of flask boil gently. When the solution becomes perfectly clear, allow it to cool. Transfer the contents of this flask to a 500-c.c. flask containing 100 c.c. of water, washing out the original flask thoroughly and adding the washings to the second flask. Alkalinize the contents of flask with 25 per cent. sodium hydrate solution (avoiding too rapid addition of alkali, in order to prevent generation of sufficient heat to liberate ammonia). Add a little talcum powder (to prevent bumping), and connect the flask with a special Fresenius bulb, with condenser attachment. Heat the contents of the flask to boiling, and collect condensed vapors in a measured quantity of *n*-10 sulphuric acid. After about 100 c.c. of fluid have distilled over, remove the end of tube from the receiving flask and then remove flame from the distilling flask. Titrate the fluid in the receiving vessel against *n*-10 sodium hydrate, using congo-red as an indicator. Calculate the amount of ammonia which the method has yielded, and from this determine the percentage of nitrogen in urine.

VI. DETECTION AND ESTIMATION OF ORGANIC CONSTITUENTS OF NORMAL URINE.

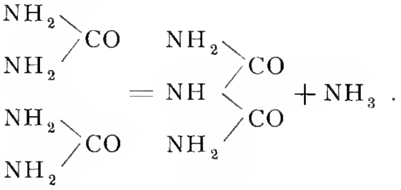
1. *Urea*,  $OC{<}^{NH_2}_{NH_2}$.—Urea is the most important nitrogenous

constituent of the urine, representing normally about 85 per cent. of the total amount of nitrogen eliminated by the kidneys.

a) *Preparation XXVIII: Preparation of urea.*—Evaporate 500 c.c. of urine to the consistency of a thin syrup. Cool and add twice the volume of nitric acid (50 per cent.). Allow to stand in a cool place over night. Filter off the crystals of urea nitrate (using suction-pump and porcelain sieve), wash them with a small amount of water containing nitric acid, and remove crystals from the filter and dissolve them in a small amount of hot water. Add to this aqueous solution, stirring constantly, a little potassium permanganate solution, until the solution of urea nitrate is colorless. Add now to this boiling, colorless solution finely divided barium carbonate until effervescence of carbon dioxide ceases and the solution becomes neutral. Evaporate over water-bath to dryness, powder the residue, and extract it with 95 per cent. warm alcohol. Filter and set filtrate aside to cool. Urea crystallizes out and should be recrystallized from warm alcohol. Examine crystals microscopically, and test their solubility in water, alcohol, and ether.

b) *Qualitative detection of urea.*

(1) Heat, slowly, a little of the dry urea in a test-tube. Notice that the urea melts and a sublimate of ammonium carbonate is formed. Continue the heating until the melted mass begins to solidify. Cool and dissolve the residue in very dilute sodium hydrate. Perform the biuret test with a portion of the solution.



(2) To remainder of the sodium hydrate solution obtained above add hydrochloric acid. Notice white precipitate of cyanuric acid.

$3CO(NH_2)_2$ (by the aid of heat) $= C_3H_3N_3O_3 + 3NH_3$

(3) To a small portion of urea dissolved in water add mercuric nitrate solution. Notice the white precipitate formed. This is the basis of the Liebig volumetric determination of urea.

(4) Dissolve a few crystals of urea in a few drops of water and add an equal volume of concentrated nitric acid. Notice precipitate. Examine it microscopically.

(5) Heat urea with sodium hydrate solution and notice the evolution of carbon dioxide and ammonia.

$$CO(NH_2)_2 + 2H_2O = CO_2 + 2NH_3 + H_2O \; .$$

(6) To a little bromine water in a test-tube add sodium hydrate solution (avoid heating). Sodium hypobromite is formed. To this solution add an aqueous solution of urea and notice the evolution of gas.

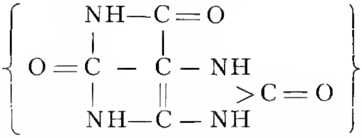
$$CO(NH_2)_2 + 3NaOBr = CO_2 + 2N + 2H_2O + 3NaBr \; .$$

This reaction is the basis of the Knop-Hüfner method of estimating urea, the nitrogen is measured directly (corrections must be made for temperature and barometric pressure) and the carbon dioxide is absorbed by the excess of sodium hydrate used in the preparation of the hypobromite solution.

c) *Quantitative estimation of urea.* — Obtain from the instructor a Doremus ureometer. The principle of this determination is that given in the previous experiment. Fill the vessel with the hypobromite solution (100 g. of sodium hydrate dissolved in 250 c.c. of water, and 25 c.c. of bromine added after the sodium hydrate solution has cooled), and add very carefully, by means of a curved pipette, 1 c.c. of urine to this solution. In injecting the urine into the solution in the ureometer, care should be taken to get the curved end of the pipette well under the curve of the bulb, and also to force the urine into the hypobromite solution with a slow, steady pressure. An evolution of gas is noticed at once. This, however, ceases in a few minutes. Read off the amount of gas and determine the percentage of urea in the urine. The degrees marked on the tube indicate, directly, the number of grams of urea in the 1 c.c. of urine used.

Make two urea determinations with your urine: one with urine obtained in the morning before breakfast, the other with urine obtained after a hearty meal. Observe the differences and explain the variations.

2. *Uric acid,* $C_5H_4N_4O_3$



.—

Although the amount of uric acid excreted is small, generally less than a gram *per diem,* yet it is probable that far more is produced and then transformed in the liver into urea. The excretion of uric acid is often, for reasons not well understood, strongly influenced by certain conditions, which cause a tendency to a deposition of uric acid in the tissues or an increase in the urine. Uric acid is derived, in part, from the nucleins of the food, and in part from the metabolism of the white blood corpuscles of the body. It is to be remembered, however, that the other purin bodies, such as xanthin and hypoxanthin, owing to their similar chemical properties, may introduce an error into the estimation of uric acid in the urine.

a) *Preparation XXIX: Preparation of uric acid.*—To 1,000 c.c. of urine, which may be concentrated by evaporation, add 100 c.c. of concentrated hydrochloric acid and allow to stand in a cool place for twenty-four hours. Decant and filter off the crystals, which are usually colored red or brown. Wash these crystals with water and dissolve them in dilute (10 per cent.) sodium hydrate solution. Decolorize this solution by boiling it with a little animal charcoal. Filter and add to the colorless filtrate strong hydrochloric acid up to a strong acid reaction. Heat the solution to boiling and set aside to cool and crystallize. Collect the crystals on filter, wash with water, and dry them on a porcelain plate. Examine crystals microscopically and test their solubility in water, alcohol, ether, acids, and alkalies.

b) *Qualitative detection of uric acid.*

(1) *Murexid test.*—Place a very small quantity of uric acid in an evaporating-dish and add a few drops of concentrated nitric

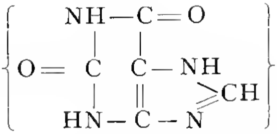
acid. Evaporate on water-bath to dryness. A yellowish or reddish residue remains. Allow the residue to cool and add a few drops of ammonium hydrate solution. Notice the reddish-purple color. If, instead of ammonium hydrate, sodium hydrate be added, the color is pure blue. Add a little water and evaporate to dryness. Notice that the color disappears. The purple color is due to formation of murexid, and the treatment with water destroys this. Xanthin and the other purin bases give this murexid test, but the color does not disappear in the latter cases on treatment with water or on heating quickly.

(2) *Schiff's reaction.*—Dissolve a little uric acid in 10 per cent. sodium carbonate solution, and place a drop of this solution on filter paper, previously moistened with silver nitrate solution. A yellowish, or sometimes black, coloration, due to formation of metallic silver, appears.

(3) Boil a solution of uric acid in sodium hydrate with a small amount of Haines's or Fehling's solution. Notice that reduction occurs with production of a whitish-gray or reddish precipitate (if the copper salt be in excess).

(4) To a sodium carbonate solution of uric acid add magnesia mixture (1 part crystallized magnesium sulphate, 2 parts pure ammonium chloride dissolved in 8 parts of water, add 4 parts strong ammonia water, and filter), and then silver nitrate solution. Uric acid is precipitated as a gelatinous magnesio-silver urate. This test may be used for the quantitative determination of uric acid.

c) Quantitative determination of uric acid (Hopkin's method).—One hundred c.c. of urine are treated with 30 g. of powdered chemically pure ammonium chloride. Shake the mixture and set aside for several hours (over night). Filter and wash the precipitate with a saturated solution of ammonium chloride. Wash the precipitate from the filter with boiling water and decompose it by addition of 5 c.c. of 25 per cent. hydrochloric acid solution. Evaporate this solution until crystals begin to separate out. Collect on filter, wash with water, alcohol, and ether, and dry in oven. Weigh the precipitate, which is pure uric acid. As uric acid is slightly soluble in water, 0.0048 g. should be added to the weight of uric acid for every 10 c.c. of wash water used.

3. *Xanthin bases:* Xanthin, $C_5H_4N_4O_2$  .—

The xanthin (alloxur or purin) bases are of great importance owing partly to their close chemical relationship to uric acid and partly to their excretion as normal constituents of the urine. These bodies are derived from two sources: first, from the nucleins of the food, giving the exogenous purin bases; and, second, from the decomposition of the nucleins of the body, giving the endogenous purin bases. It is to be remembered that these bodies show many of the reactions of uric acid, and may therefore introduce errors in the estimation of this latter substance.

a) *Preparation* (*Salkowski*).—Evaporate 1 L. of urine to one-half its bulk; supersaturate this evaporated urine with strong ammonia water. Add a slight excess of 2 per cent. silver nitrate solution (until no further precipitation occurs). Suspend this precipitate in boiling water and pass hydrogen sulphide as long as a precipitate forms (the solution should be heated while the gas is passing). Filter off the sulphide of silver and evaporate the filtrate to dryness. Treat the dried residue with hot 3 per cent. sulphuric acid, which dissolves the xanthin bases and leaves the uric acid behind. Filter, saturate the filtrate with strong ammonia water, and add 2 per cent. silver nitrate solution. This precipitates all of the xanthin bases. These may be separated and isolated, but the details will not be given here (see "Muscle," chap. 4). Filter off this precipitate and dry on porcelain plate. Test solubility in water, alcohol, acids, and alkalies.

b) *Qualitative detection of xanthin bases.*

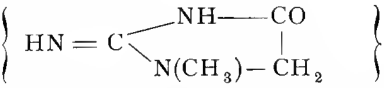
(1) *Weidel's reaction for xanthin.*—Heat a little of the crystalline deposit obtained above with a few drops of chlorine water and one or two drops of nitric acid, and evaporate to dryness. Place the dish containing this residue under a large beaker and allow the fumes of ammonia to fill the inverted beaker. Observe the red or purple-violet color produced. This reaction is not given by hypoxanthin.

(2) *Murexid test.*—Perform this test as directed under uric acid, and observe variations in case of xanthin bases.

(3) *Kossel's test for hypoxanthin.*—Place some of the crystals obtained above in a test-tube, pour on them a few (5) cubic centimeters of dilute (10 per cent.) hydrochloric acid, and add a little granulated zinc. After the evolution of hydrogen has continued for a little time, add an excess (10 c.c.) of 10 per cent. sodium hydrate solution. Observe the ruby-red and then brownish-red coloration.

c) *Quantitative determination of xanthin bases* (*Salkowski*).—Mix 500 c.c. of albumin-free urine with 50 c.c. of magnesia mixture, and add 50 c.c. of strong ammonia water. After a few minutes filter through a dry folded filter, and add to filtrate 40 c.c. of 3 per cent. watery solution of silver nitrate or 60 c.c. of ammoniacal silver nitrate solution. Allow the gelatinous precipitate to settle for an hour, bring it upon a smooth filter, and wash it silver-free with water. Bring the precipitate into a flask, dilute largely with water, and add a few drops of hydrochloric acid. Pass a stream of hydrogen sulphide to remove the silver (keeping solution warm, while gas is passing through). Filter hot, wash the precipitate thoroughly, and evaporate the filtrate to dryness. Treat this dry residue with 25--30 c.c. of dilute (3 per cent.) sulphuric acid and heat to boiling. Allow the fluid to cool and to stand for twenty-four hours. Filter off the undissolved uric acid, and wash this with dilute sulphuric acid. Saturate the filtrate, together with wash water, with ammonia, and precipitate with silver nitrate solution. Filter through an ash-free filter, and wash the precipitate free of chlorine, silver, and sulphuric acid. Dry the precipitate on the filter, ignite in a porcelain crucible, and dissolve the silver in chlorine-free acid. Estimate the amount of silver by titration with 0.02 *n* potassium sulphocyanate solution, according to Volhard.

4. *Kreatinin*, $C_4H_7N_3O$



.—

Kreatinin is present in the urine only in small amounts (0.6--1.3 g. in twenty-four hours). The amount present is largely dependent on the food and on the amount of muscular decomposition taking place.

a) *Preparation XXX: Preparation of kreatinin* (Neubauer).—Alkalinize 200 c.c. of urine with milk of lime, then add calcium chloride solution until all the phosphates are precipitated. Filter, wash with water, and evaporate the filtrate (after faintly acidifying it with acetic acid) to consistency of a syrup. To the syrup while still warm add 50 c.c. of 95 per cent. alcohol, transfer to a small beaker, cover with glass plate, and allow to stand in a cool place over night. Filter through a small filter, wash the precipitate with alcohol (adding the washings to original filtrate), evaporate the filtrate to 40 c.c., and allow it to cool. Add ½ c.c. of saturated alcoholic zinc chloride solution, stir thoroughly, cover beaker with glass plate, and allow to stand for two or three days. Collect the crystals of kreatinin zinc chloride and wash them with alcohol. Examine crystals microscopically.

b) *Qualitative detection.*

(1) *Weyl's reaction.* — See "Kreatinin" under "Muscle," (chap. 4).

(2) *Jaffe's test.*—To a portion of the urine or of an aqueous solution of kreatinin zinc chloride add a cubic centimeter of aqueous solution of picric acid and then a few drops of dilute sodium hydrate. Notice the deep-red color.

(3) Boil a few cubic centimeters of Fehling's solution with a little of the urine or solution of salt obtained above. Notice the odor of ammonia and the decolorizing of the solution. This test is of importance in connection with the testing of urine for sugar by reduction tests.

c) *Quantitative determination.*—Proceed as in the preparation, and continue as follows: Bring the crystals of kreatinin zinc chloride upon a dried and weighed filter. Wash the crystals with small amounts of 95 per cent. alcohol until the washings are colorless and show no further reaction for chlorine. Dry the crystals on filter at 100° C. and weigh. Calculate amount of kreatinin from the weight of the salt.

5. *Coloring matter of normal urine.*—The yellow color of normal urine depends apparently upon several coloring matters not yet isolated (called by Garrod urochrom). Several pigments, however, appear in urine in conditions which are more or less normal. These pigments are derived from the biliary color-

ing matters (see table under "Blood," chap. 4). Among these pigments we have urobilin, uroerythrin, and urobilinoidin.

a) *Preparation XXXI: Preparation of urobilin* (*Jaffe*). —Add to the urine a solution of basic lead acetate, filter, wash the precipitate with water, dry at ordinary temperature, boil it with alcohol, and decompose it, when cold, with alcohol containing sulphuric acid. The filtered solution is diluted with water, saturated with ammonia, and then treated with zinc chloride solution. Wash this latter precipitate free from chlorine by water, boil it with alcohol, dry, dissolve in ammonia, and precipitate this solution with lead acetate. Wash this precipitate with water, boil with alcohol, decompose with alcohol containing sulphuric acid, filter, and mix the alcoholic filtrate with one-half volume of chloroform. Dilute with water and shake gently. The urobilin is taken up by the chloroform. Wash this once or twice with water and distil. Purify the urobilin, which remains behind, by means of ether.

b) *Qualitative detection.*

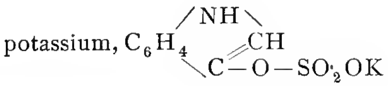
(1) Dissolve some urobilin in alcohol and add some sodium hydrate solution. Notice the brownish-yellow coloration. Now acidify this solution with hydrochloric acid and notice the change of color to brown, reddish-yellow, or rose-red.

(2) Dissolve some urobilin in ammonium hydrate and add a cubic centimeter of zinc chloride solution. Notice the green fluorescence. Examine spectrum of this solution. See Plate I.

c) *Quantitative determination* (*G. Hoppe-Seyler*). — One hundred c.c. of urine are acidified with sulphuric acid and saturated with ammonium sulphate. Allow to stand for two hours and collect the precipitate on a filter, wash with a saturated solution of ammonium sulphate, and extract several times with equal parts of alcohol and chloroform. The filtered solution is treated with water in a separatory funnel until the chloroform separates well and becomes clear. Draw off the chloroform solution and evaporate it in a weighed beaker on water-bath. Dry the residue at 100° C. and extract it with ether. Filter the ethereal extract, dissolve residue on filter, in alcohol, and transfer it to the weighed beaker. Evaporate, dry, and weigh.

6. *Indican*

{indoxyl sulphate of potassium,



}.—

The indol formed during the process of intestinal putrefaction is oxidized to indoxyl in the blood. This latter enters into combination with sulphuric acid and is eliminated in the urine as indican.

a) *Qualitative detection.*

(1) *Jaffe's test.*—To 10 c.c. of urine add an equal volume of concentrated hydrochloric acid and then 1 c.c. of chloroform. Add to this mixture, drop by drop, a saturated solution of calcium hypochlorite (bleaching powder) until the maximum of blue color is obtained. (Care should be taken not to add a large excess of the hypochlorite solution, as the oxidation will proceed too far.) Shake the tube gently and observe the blue color of the chloroform.

(2) *Obermayer's test.*—Precipitate the urine with lead acetate solution. Add an equal volume of concentrated hydrochloric acid to filtrate and 1 c.c. chloroform. Now add a few drops of ferric chloride solution. Shake and observe blue coloration of chloroform.

b) *Quantitative determination.*—Proceed as in Obermayer's test. Shake out the fluid several times with chloroform, transfer chloroform to a weighed dish, and evaporate at temperature a little below boiling-point of chloroform (61.2°). Dry residue at 100° C., cool, and weigh. Calculate amount of indican from weight of indigo-blue.

VII. DETERMINATION OF THE ACIDITY OF THE URINE.

The acidity of the urine is due, for the most part, to the presence of sodium dihydrogen phosphate, partly to the presence of free acid and excess of uric acid. The phosphates present in the urine are of two kinds: the mono- and dihydrogen phosphates. The former are precipitated by barium chloride, while the latter are not. These facts are the basis of Freund's method of determining acidity of urine (look up in text-book).

For ordinary clinical purposes the following method is approximately correct, although an accurate determination is impossible by this method.

Titrate 10 c.c. of urine with *n*-10 sodium hydrate solution, using phenol-phthalein as an indicator. Calculate the acidity in terms of oxalic acid.

VIII. DETECTION AND ESTIMATION OF ABNORMAL CONSTITUENTS OF URINE.

1. *Proteids.*—The appearance of slight traces of proteid in urine of apparently healthy persons has been observed in many cases. In diseased conditions proteid occurs in the urine in a variety of cases. The four proteids of the blood, namely, serum albumin, serum-globulin, fibrinogen (as fibrin), and hæmoglobin, are met with in the urine in various pathological conditions. Other proteids, such as egg-albumin, are sometimes met with upon the free ingestion of eggs as food, and, under certain conditions, certain proteids (such as proto- and deutero-albumose) and peptone are also present. Mucin is found in practically all urines, normal or abnormal, although the quantity is variable.

a) *Serum albumin.*—The term "albuminuria" is usually applied to the condition when this proteid is present in the urine.

(1) *Heat test.*—Heat a portion of the clear filtered urine to boiling. If it remains clear and the reaction is acid, no albumin is present. If it becomes cloudy, this cloudiness is due to albumin or calcium phosphate. To determine which is the cause, acidulate with a few drops of acetic acid. If the urine becomes clear, the precipitate first noticed is the phosphate; if it remains turbid, the precipitate is albumin. It is to be remembered that, if the proteid be very slight in amount, and especially if the urine was originally alkaline, the proteid will remain in solution, owing to the formation of acid albumin. If not enough acid is added, the precipitate of phosphate may not dissolve, and hence a wrong idea may be formed. For these reasons it is better to add the acid after boiling.

(2) *Heller's nitric acid test.*—To a few cubic centimeters of filtered urine in a test-tube add 3–4 c.c. of concentrated nitric acid, in such a manner as to form a distinct layer of acid below the urine (it is to be remembered that, on addition of a small amount of nitric acid, a compound, soluble on boiling, is formed). If albumin be present, a distinct white ring is observed just at the point of contact of the two liquids (a red or reddish-violet transparent ring is always obtained with normal urine, while, with urines containing abnormal coloring matters, various colored

rings are observed, each being more or less significant of certain substances, which will be discussed later). If the urine be rich in urea, a white or yellowish crystalline ring appears at junction of the liquids, but will not appear if the urine be previously diluted. If an excess of uric acid be present in the urine, we observe, in a few minutes, a distinct white ring in the urine about 1--2 cm. above the point of contact of acid and urine. If mucin is present, a diffuse cloud appears throughout the urine, or a distinct ring above that of uric acid may be observed. If albumoses be present, a white ring appears at the junction of the two liquids, but this ring will disappear on boiling, while that due to albumin will become more compact.

If the ring at junction of the acid and urine be due to resinous bodies excreted by the urine, this ring will disappear on addition of ether or alcohol, and shaking, while that due to albumin is undissolved. This test is usually applied by combining it with the heat test. If the various possibilities of this reaction be known, it is the most reliable test for albumin in the urine.

(3) *Purdy's test.*—Fill a clean test-tube two-thirds full of filtered urine; add to this one-sixth of its volume of a saturated sodium chloride solution. Now add five to ten drops of acetic acid and boil the upper part of the contents of the test-tube. If albumin be present, a coagulum will appear in the boiled portion of the tube.

This test has the advantage of being very delicate and at the same time of preventing the precipitation of other proteids, such as albumoses, which may be present.

(4) *Ferrocyanide test.*—Add to the urine acetic acid up to approximately 2 per cent. acidity, and then add potassium ferrocyanide solution, drop by drop. If albumin is present, a white cloud or ring will appear at point of contact, or, if the solution has not been added carefully, a diffuse cloudiness will be observed. This is a very delicate test, but has the disadvantage of precipitating all forms of proteids.

(5) *Trichloracetic acid test.*—By means of a pipette place 1 c.c. of an aqueous solution of trichloracetic acid in the bottom of

a test-tube containing a few cubic centimeters of clear filtered urine. A white ring will form at the zone of contact, if albumin be present. This test gives precipitates with all forms of proteids, but all disappear on heating, with exception of serum albumin.

(6) *Picric acid test.*—Place a few cubic centimeters of aqueous picric acid solution in a test-tube. Carefully add a little clear filtered urine, and notice white contact zone, if albumin be present. On heating, albumin collects into balls, which rise to surface. This test gives positive reaction with all forms of proteids, but not if the urine be boiled. Alkaloids are also precipitated, if present.

(7) *Biuret test.*—Apply this test in usual way and note result.

b) *Serum-globulin.*—Make a few cubic centimeters of urine alkaline by addition of ammonium hydroxide. Filter off the precipitated phosphates. Neutralize the filtrate with acetic acid and treat it with an equal volume of a saturated solution of ammonium sulphate. A white flocculent precipitate is formed in presence of globulin. If the urine be rich in urates, a precipitate consisting of ammonium urate may separate out after some time, and should not be mistaken for globulin.

c) *Hæmoglobin.*—To a few cubic centimeters of urine in a test-tube add a few drops of acetic acid and examine the solution with the spectroscope, noting the two absorption bands of oxyhæmoglobin (if blood is present). Now add ammonium sulphide, cork the tube, and shake well. Allow to stand a short time, and examine solution with spectroscope. Note the one broad absorption band of hæmoglobin.

d) *Fibrinogen* (present as fibrin). — This proteid usually occurs in the urine in the form of clots or shreds. These are dissolved by warming on water-bath in 1 per cent. sodium hydrate solution or 5 per cent. hydrochloric acid solution. On cooling, serum albumin may be detected by tests given above.

e) *Mucin.*—Mucin, as such, is said to appear in small quantities in normal urine, but is present more frequently as a consequence of catarrhal affections. In most cases, what is usually termed mucin is doubtless a nucleo-proteid similar to mucin. According to Mörner, we have three classes of mucin-like substances: (1) chondro-albumins (albumin+chondroitin sulphuric acid), (2) nucleo-proteid (albumin+nucleinic acid), (3) taurochol albumin (albumin + taurocholic acid).

Dilute carefully filtered urine with water (to prevent precipitation of uric acid and to reduce the solvent action of the salts on the mucin), and add an excess of concentrated acetic acid (drop by drop). The occurrence of a precipitate indicates a mucin-like body. If the urine contains albumin, this must be removed before applying test for mucin.

f) *Albumoses.*—Test the urine for albumin. If it is present, acidify strongly with acetic acid, add an equal amount of a saturated sodium chloride solution, boil, and filter. On cooling, if albumoses be present, a cloudiness appears, disappearing on heating and reappearing on cooling. Apply the biuret test to filtrate, and note result.

g) *Peptones.*—To 50 c.c. of urine add 5 c.c. hydrochloric acid and precipitate with phospho-tungstic acid, heating the mixture over a free flame. Decant the supernatant liquid from the precipitate, which forms a resinous mass. Wash the precipitate with water by decantation. Cover the precipitate with 8 or 10 c.c. of distilled water and treat with 1 c.c. of sodium hydrate solution. Upon shaking the beaker the precipitate dissolves, with formation of a blue solution. Heat this solution over a free flame until the blue color turns to a dirty grayish-yellow (the solution usually becomes turbid at the same time, but may remain clear). As soon as this point is reached, place the solution in a test-tube, allow to cool, and add a few drops of copper sulphate solution. If peptones be present, a bright-red color appears. If albumin or mucin be present, they must be removed before this test can be applied. By this method 0.015 g. of peptone per 100 c.c. may be detected.

h) *Quantitative estimation of proteids.*—In this determination only the albumin will be considered, as it is the most important clinically. The quantity of proteids is, in most cases, less than 0.5 per cent., rarely 1 per cent., very rarely 5 per cent.

(1) *Coagulation method* (*Scherer*).—Treat 50 c.c. of urine, after it has been brought to boiling, with acetic acid. Add the acid slowly. Stir constantly and filter the boiling solution. Test the filtrate with nitric acid for proteids. If a positive reaction occurs, not enough acetic acid has been added. If the test is negative, wash the precipitate with water, alcohol, and ether. Dry and weigh this washed precipitate.

(2) *Esbach's method* (*approximate estimation*).—The acidified urine is poured into a specially graduated Esbach tube up to the mark "urine." Add the reagent (2 per cent. citric acid + 1 per cent. picric acid) up to mark "reagent," close the tube with a rubber stopper, and shake carefully. Allow the tube to stand twenty-four hours and then read off amount of albumin. The reading gives directly the amount of proteid in 1,000 parts of urine. Care should be taken to allow the tube to stand in a room whose temperature is constant.

2. *Carbohydrates.*—The term "glycosuria" is usually applied to the condition in which glucose is found in the urine. The carbohydrates which may appear in the urine besides glucose are lævulose, lactose, maltose, pentose, dextrin, and animal gum. As glucose is the most common and most important, it alone will be discussed.

a) *Qualitative detection of glucose* (*d-glucose*).

(1) *Trommer's test.*—In every case remove albumin, if present, before testing for sugar by acidifying, boiling, and filtering the urine. Strongly alkalinize a few cubic centimeters of urine with sodium hydrate, and then treat it with a few drops of 5 per cent. copper sulphate solution (add the copper sulphate, drop by drop until the cupric hydroxide formed is no longer dissolved). Carefully boil the mixture, when a yellowish or reddish-yellow precipitate of cuprous oxide is formed, if sugar be present.

This test is open to two objections: (1) If the mixture be not boiled, it is not sensitive, detecting sugar only when present in relatively large amount. (2) If it be boiled, other substances, such as uric acid, kreatinin, allantoin, mucin, lactose, maltose, pyrocatechin, hydroquinone, bile-pigments, glycosuric acid, and glycuronic acid, may cause this reduction. Addition of preservative agents, such as chloral or formaldehyde, may cause a reduction.

(2) *Fehling's test.*—A modification of above, consisting in the addition of Rochelle salt (sodium-potassium tartrate), to hold the cupric hydrate in solution.

(3) *Haines's test.*—Another modification of Trommer's test, using glycerine to keep the cupric hydrate in solution. This solution has the advantage over Fehling's solution that it keeps for a long time after mixing. Both this and the preceding test are open to objections raised under Trommer's test.

In using Haines's test always boil the solution first and then add not more than ten drops of the suspected urine. If no reduction occurs, add ten drops more, boil, and set aside for a few minutes. If no reduction occurs, the *absence* of sugar is indicated, although a reduction does not, of necessity, indicate the *presence* of sugar.

(4) *Böttger's bismuth test.*—See "Carbohydrates" (chap. 1). Sulphur compounds (hence albumin), if present, give a black color reaction with this test. Certain drugs, such as quinine and eucalyptus, will also cause a reduction.

(5) *Fermentation test.*—A small piece of ordinary yeast is shaken with some of the suspected urine, and a fermentation-tube filled with the mixture. Fill another tube with normal urine (which does not show any reducing action on copper solutions), to which a piece of yeast is added (to be used as a control). Set both tubes aside in the desk until the next exercise. Note difference in amount of gas in the two tubes.

This test has the advantage that the other substances, which reduce the copper and bismuth solutions, do not ferment. This should always be used as a confirmatory test whenever a reduction test has been observed.

(6) *Phenyl-hydrazine test.*—See "Carbohydrates" (chap. 1). It is to be remembered that only the yellow needle-shaped crystals, often interlacing, having a melting-point of 204° C., are characteristic of glucose. Lævulose, if present, is the only other carbohydrate which will give this same phenyl glucosazone.

(7) *Polarization test.*—Test the effect of the suspected urine upon the plane of polarized light (see instructor).

Glucose has a definite value, which is dependent, of course, on the concentration and temperature.

$$[\alpha]_D = +52.5$$

b) *Quantitative determination of glucose.*—The methods used here are based essentially upon the qualitative tests just given. The most satisfactory estimation is, of course, that given by polarimetric examination. A good clinical method is the one based upon the reduction of copper solutions by sugar. For this purpose we may use Fehling's, Haines's, or Purdy's solutions. The former will be given in this estimation. The Fehling's solution is made up as follows: 34.65 g. of pure copper sulphate is dissolved in ½ L. of water. Potassium sodium tartrate (173 g.) and potassium hydroxide (125 g.) dissolved in 500 c.c. of water. The copper solution and the alkaline tartrate solution are kept in separate bottles, and mixed in equal parts for use. The principle of the test consists in the reduction of cupric oxide (in solutions of definite strengths) by grape sugar; the blue coloration disappears by addition of a definite amount of sugar. With this solution, 10 c.c. are reduced by 0.05 g. of sugar.

Mix in an Erlenmeyer flask 10 c.c. of the copper sulphate solution, and add 10 c.c. of the alkaline Rochelle salt solution and 30 c.c. of water. Bring this mixture to boiling-point. Fill a burette with the suspected urine (which should be proteid-free and should be diluted five to ten times according to specific gravity of the urine), and allow it to flow, drop by drop, into the boiling solution in the Erlenmeyer flask, until the blue color completely disappears. To facilitate the settling of the cuprous oxide formed, a little calcium chloride solution may be added (Munk). A few seconds must be allowed to elapse between the addition of each drop of urine, in order to permit cuprous oxide to settle. The number of cubic centimeters of urine required to discharge the blue coloration of the 10 c.c. of copper sulphate solution used contain 0.05 g. of glucose. If the urine has been diluted, allowance must be made for this by multiplying the figure obtained by the amount of dilution.

Calculate the percentage of sugar in the urine.

c) Lævulose, cane sugar, maltose, and lactose may be detected by tests learned in chap. 2. Pentoses are detected by Tollen's test. Dissolve a little phloroglucin in 5 c.c. of concentrated hydrochloric acid (heat to facilitate solution, and allow to cool). Divide the cooled solution into two parts. To one add ½ c.c. of the suspected urine. To the other add ½ c.c. of normal urine. Place both tubes in water-bath. If pentoses are present, a deep-red coloration is observed, while the color is little changed from the normal, if they be absent.

3. *β-oxy butyric acid* (CH_3—CHOH—CH_2—COOH).—This acid is always accompanied by diacetic acid in the urine, although the reverse is not the case. It is detected as follows:

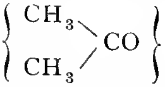
a) Allow the suspected urine to ferment with yeast. Examine the filtered solution with polarimeter. If solution is still lævo-rotatory, the presence of the β-oxy butyric acid is probable.

b) *Külz's test.* — Allow the urine to ferment with yeast. Evaporate the fermented urine to a syrup, add an equal volume of concentrated sulphuric acid, distil (without previous cooling of mixture). Collect distillate and allow to cool. Crystals of α-crotonic acid (m.p. + 72° C.) separate. If no crystals appear, shake distillate with ether, and test the melting-point of residue obtained after evaporating the ether.

4. *Diacetic acid* (C_2H_3O—CH_2—COOH).—The urine may be tested for diacetic acid directly or this acid may be removed as noted under acetone. It may be detected by the following tests:

Gerhardt's reaction.—To a few cubic centimeters of urine, or of ethereal solution of diacetic acid obtained by shaking out urine, add a few drops of ferric chloride solution.Should a precipitate of phosphates appear, filter, and add a little more ferric chloride solution to filtrate. Note occurrence of Bordeaux-red color in presence of diacetic acid. Heat this solution and observe result. Explain.

Antipyrin, if excreted in the urine, gives this same red color with ferric chloride, but the color is permanent on heating.

5. *Acetone*  $\left\{\begin{matrix}CH_3\\CH_3\end{matrix}\right\rangle CO\Big\}$.—An abundant elimination of acetone, diacetic acid, and β-oxy butyric acid is observed, especially in diseases which are associated with great destruction of body-proteid.

Detection.—In applying tests for acetone in the urine, diacetic acid should be tested for previously, as this breaks up into acetone on heating. If diacetic acid is present, acidify urine with sulphuric acid and shake out with ether. The acid goes into solution, leaving acetone behind. In absence of diacetic acid, the urine may be tested directly for acetone. A better method, however, is the following: Distil at least 250 c.c. of urine faintly acidified with sulphuric acid. Most of the acetone is found in the first 10--20 c.c. of the distillate. This distillate is then tested for acetone by following tests:

a) *LeNoble's test* (*Legal's test*).—To a few cubic centimeters of urine, or of distillate obtained above, add a few drops of a dilute sodium nitro-prusside solution and then a little dilute sodium hydrate. A ruby-red color, later changing to yellow, appears. Saturate this red solution with acetic acid and notice the change to carmine or reddish-purple.

Kreatinin also gives this test, but on saturation with acetic acid the color is first yellow and then greenish-blue. If ammonium hydrate be used instead of sodium hydrate, kreatinin does not give the reaction.

b) *Reynold's test.*—Treat a few cubic centimeters of urine with a small amount of freshly precipitated yellow oxide of mercury (prepared by precipitating a solution of mercuric chloride with an alcoholic solution of potassium hydroxide, and filtering). Filter and add to the filtrate a few drops of ammonium sulphide. Notice the black color, due to sulphide of mercury, which appears if acetone be present.

c) *Lieben's iodoform test.*—To a few cubic centimeters of urine add sodium hydrate and then some iodo-potassium-iodide, and warm slightly. Notice the yellow crystals. Filter and observe the odor of these crystals. Examine them microscopically. What are they? Alcohol, among other organic substances, gives this reaction.

6. *Bile.*

a) *Biliary acids.*—Albumin, if present, must first be removed.

Pettenkoffer's test.—Mix 10 c.c. urine with 1 c.c. concentrated sulphuric acid (taking care that the temperature does not rise above 70° C.). Add, drop by drop, a 10 per cent. solution of cane sugar, stirring constantly. A red coloration indicates bile-acids.

b) *Biliary pigments.*

(1) *Gmelin's test.*—To 10 c.c. of urine in a test-tube add a few cubic centimeters of yellow nitric acid, in such a manner as to form distinct layers (hold the test-tube at an angle and allow the acid to flow slowly down the side). A play of colors (green, blue, violet, red, yellow) is noticed. The initial green is indispensable as an indication of bile pigments.

(2) *Rosenbach's modification of above test.*—Filter 10 c.c. urine through a small filter. Pour a drop of yellow nitric acid upon filter. A pale-yellow spot surrounded by colored rings is observed in presence of bile pigment.

(3) *Huppert's test.*—Treat 20 c.c. of urine with lime-water. Notice the precipitate which carries with it any bile pigments present. Filter, wash the precipitate with water, acidify this precipitate with acetic acid, and shake in a test-tube with chloroform. The bilirubin is taken up by the chloroform, which is, hence, colored yellow while the biliverdin is taken up by the acetic acid with a green coloration.

(4) *Ultzmann's test.*—Mix 10 c.c. of urine with 3 c.c. of sodium hydrate solution. Supersaturate the mixture with hydrochloric acid. Notice the emerald-green color.

(5) *Hay's test.*—Add to urine suspected of containing bile a little "flowers" of sulphur. In presence of 1 part of bile to 50,000, the sulphur sinks to bottom of test-tube. Other substances, such as phenol and aniline, which lower the surface tension of the urine will bring about this reaction.

7. *Blood.*—The presence of blood in the urine is usually shown by the blood-coloring matters or proteid compounds. These latter have been already discussed. The former are shown by following tests.

a) *Oxidation test.*—Mix in a test-tube equal parts of tincture of guaiacum and of ozonized turpentine. Allow this to flow carefully down the side of another tube containing urine, so as to form a distinct layer above the urine. Notice the white ring changing to blue. Shake the mixture and notice the blue coloration.

b) *Heller's test.*—Alkalinize some urine with sodium hydrate and boil. Notice the precipitate of earthy phosphates, which is colored red if blood is present. If the urine contains a large amount of coloring matter, this red coloration may not be easily noticed. In this case, filter, dissolve the precipitate in acetic acid. Notice the red coloration of solution, gradually disappearing on exposure to air.

c) *Spectroscopic test.*—Examine some of the clear or slightly "smoky" filtered urine with spectroscope. Refer absorption bands to those under work on "Blood" (chap. 4).

d) *Struve's test.*—Alkalinize the urine with sodium hydrate, add solution of tannic acid, filter, and test the precipitate for hæmin crystals as under "Blood" (chap. 4).

IX. URINARY SEDIMENTS.

Urinary sediments are classified in two divisions: (A) chemical or non-organized, and (B) anatomical or organized sediments. The non-organized sediments exist in solution in normal urine and appear as deposits under conditions of excessive formation, excessive excretion, or of alterations in the urine affecting its solvent powers. The chief chemical sediments are uric acid, urates, calcium oxalate, cystin, leucin, tyrosin, xanthin, phosphates, fat, and fatty acids.

The organized sediments are usually foreign substances and are not met with in normal urine. They consist of pus-corpuscles, blood, renal casts, infusoria, spermatozoa, fungi, and epithelial cells.

The methods of examining urinary sediments are both chemical and microscopical, each being confirmatory of the other.

A. *Non-organized sediments.*

1. *Those appearing in acid urine.*

a) *Uric acid* ($C_5H_4N_4O_3$).—This occurs as a sediment in the urine under three conditions of the urine: (1) great concentration, (2) high acidity, and (3) low temperature. The deposit differs from others in possessing a deep-yellow or orange-red color. The primary form of the uric acid crystal is that of the rhombic prism. Modifications of this, in form of square plates, cubes, or ovoids, are sometimes noticed. The crystals may group themselves together in rosettes or fan-shaped groups. Other irregular forms, such as dumb-bells or whetstone crystals, are often observed. See Plate IV

Confirm presence by murexid test (see above).

b) *Sodium acid urate* ($C_5H_3NaN_4O_3$).—Occurs only in acid urine and forms the bulk of the "brick-dust deposit" or "sedimentum lateritium" found when urine has cooled. The deposit is usually in the form of irregular amorphous granules of a brownish or pink color. Occasionally the sediment may be distinctly crystalline, occurring as star-shaped, fan-shaped, or needle-like crystals. See Plate V.

c) *Potassium acid urate* ($C_5H_3KN_4O_3$).—Occurs only as a granular, amorphous deposit. Owing to its greater solubility, it does not form as large an amount of the brick-dust deposit as does the sodium salt. See Plate V.

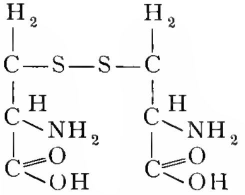
These two latter sediments of acid urates differ from other deposits in disolving if the urine be heated.

d) *Calcium oxalate* (CaC_2O_4).—This is met with in acid or alkaline urine, usually in the former. If it occurs in acid urine, it is associated with uric acid; if in alkaline urine, with the triple phosphate. The deposit is a colorless crystalline one having two distinct forms: First, octahedral crystals (four-sided pyramids lying base to base); viewed from the side these appear as squares crossed by two sharp lines, giving the "envelope" crystal. Second, dumb-bell crystals (ovoid or circular disks with round margins depressed at the centers). See Plate VIII.

Confirm presence of these by dissolving them in hydrochloric acid, alkalinizing with ammonium hydrate, and precipitating with ammonium oxalate.

e) *Cystin,*

$(C_3H_6NSO_2)_2$ or

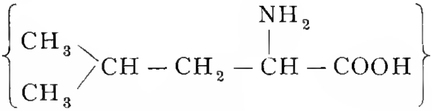


.—

This appears usually in weakly acid or alkaline urine. It crystallizes in two forms: First, six-sided tablets having an opalescent luster and sometimes traced with fine lines of secondary crystallization; second, four-sided square prisms lying separately or in stellate forms. Confirm its presence by tests learned under "Cystin" (chap. 2). See Plate XII.

f) *Leucin,*

α-amido iso butyl acetic acid

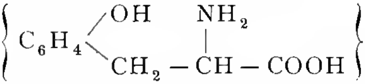


.—

Occurs as a sediment in form of yellowish, highly refracting spherules, with alternating light and dark concentric layers. It may crystallize, when in pure state, in scales or rosettes of irregular shapes. See Plate X.

Confirm its presence by tests under "Leucin" (chap. 2).

g) *Tyrosin,* p-oxy phenyl α-amino propionic acid,



.—

This substance crystallizes in form of fine colorless needles arranged in sheaf-like collections or rosettes. Confirm its presence by tests under "Tyrosin" (chap. 2). See Plate XI.

h) *Xanthin.*—This is of importance only when it appears in form of urinary calculi. It crystallizes in whetstone-shaped crystals which resemble those of uric acid, from which latter substance it is differentiated by its solubility on heating and its solubility in hydrochloric acid. Confirm its presence by tests under "Xanthin" (chap. 4). See Plate IX.

i) *Neutral calcium phosphate* ($CaHPO_4 . 2H_2O$). — Found only in weakly acid or neutral urine. It is quite rare as a sediment, crystallizing in colorless needles, which group themselves together with their points in a common center.

2. *Those appearing in alkaline urine.*

a) *Ammonium urate* ($C_5H_3(NH_4)N_4O_3$).—This sediment occurs most frequently in alkaline urine along with amorphous calcium phosphate and triple phosphate crystals. It is the only urate deposit found in alkaline urine, but may occur in neutral urine. It appears as a crystalline deposit of dark-brown spherical masses studded with fine spiculæ, from which fact the name of "thorn-apple" crystals has been given to them. Occasionally dumb-bell forms are seen. Confirm presence of this by adding sodium hydrate and heating. Ammonia vapors are given off. See Plate VI.

b) *Calcium tri-phosphate* ($Ca_3(PO_4)_2$). — This is usually found as a white amorphous flocculent deposit arranged in irregular patches.

Confirm by dissolving in nitric acid and precipitating with ammonium molybdate.

c) *Magnesium phosphate* ($Mg_3(PO_4)_2$). — Found very rarely. It occurs in large, long, rhombic plates. Confirm by dissolving in acetic acid and adding sodium dihydrogen phosphate.

d) *Magnesium-ammonium phosphate* ($Mg(NH_4)PO_4$), triple phosphate.—The appearance of this substance in the urine is essentially characteristic of ammoniacal urine. It may, very rarely, appear in amphoteric urine when ammonium salts are present in large amounts. The crystals appear as triangular prisms or "coffin-lid" crystals. These may be shortened in form of squares, or one or more corners may be absent. A second form of this triple phosphate is that of a star-shaped feathery crystal with points not unlike fern leaves. See Plate VII.

B. *Organized sediments.*

1. *Epithelial cells.*—See Plate XIII.

a) Small, round cells, spheroidal and finely granular with a single large nucleus.

b) Columnar form.

c) Squamous form.

d) Cylindrical form.

2. *Blood corpuscles.*—Biconcave disks of yellow color. May form rouleaux. Distinguished from pus corpuscles by absence of cell contents and visible nuclei. See Plate XIV. Confirm by chemical tests for blood.

3. *Leucocytes—pus.*—Circular, pale, finely granulated disks. Contain distinct nuclei. Add acetic acid and notice the change in appearance. Confirm presence of pus as follows: Add to the suspected urine a few cubic centimeters of sodium hydrate solution. If pus be present, a gelatinous, ropy coagulum is noticed. See Plate XV.

4. *Casts.*—See Plates XVI--XX.

a) Those consisting of anatomical elements.

(1) Red blood corpuscles.

(2) Leucocytes.

(3) Epithelial cells.

(4) Bacteria.

b) Products or broken-down elements of anatomical elements.

(1) Granular casts.

(2) Fatty casts.

c) Clear casts.

(1) Narrow hyaline casts.

(2) Broad casts.

(3) Composite casts.

d) *Cylindroids.*—Long, wavy, ribbon-like structures, which often divide or subdivide at ends with diminishing diameters. Ends may be folded or cork-screw shape. Rarely have cellular elements attached.

5. *Spermatozoa.* See Plate XXI.

6. *Tissue elements.*—Fragments of tumors.

7. *Bacteria.*

8. *Animal parasites.—Echinococcus, Distoma hæmatobium, filaria sanguinis hominis.*

X. URINARY CALCULI.

Concretions of a more or less hard and dense character are prone to form in the urinary passages. These bodies are termed, according to size and location, sand, gravel, stone, and calculi.

HELLER'S TABLE FOR EXAMINATION OF URINARY CALCULI.

On heating the powder on platinum foil it—									
Does not burn.				Does burn.					
The powder when treated with hydrochloric acid—				With flame—			Without flame—		
Does not effervesce.			Effervesces.	Flame yellow and continuous. Odor of burned feathers. Insoluble in alcohol and ether. Soluble in potassium hydrate on heating. Precipitated from alkaline solution by acetic acid with generation of hydrogen sulphide.	Flame pale yellow, continuous. Odor of resin or shellac on burning. Powder soluble in alcohol and ether.	Flame pale blue, burns a short time. Peculiar sharp odor. The powder dissolves in ammonia, from which six-sided plates separate on the spontaneous evaporation of ammonia.	Does not give the murexid test. The powder dissolves in nitric acid without effervescence. The dried yellow residue becomes orange on addition of alkali, red if heat be applied.	The powder gives the murexid test.	
The gently heated powder with hydrochloric acid—								The powder when treated with potassium hydrate gives—	
The powder when moistened with a little potassium hydrate—		Effervesces.						A strong ammonia reaction.	No noticeable reaction.
Abundant ammonia. The powder dissolves in acetic acid or hydrochloric acid. This solution gives a crystalline precipitate with ammonia.	No ammonia, or, at least, only traces of it. Powder dissolves in acetic acid or hydrochloric acid. This solution gives an amorphous precipitate with ammonia.								
Triple phosphate, mixed with an unknown amount of earthy phosphates.	Magnesium and calcium phosphates.	Calcium oxalate.	Calcium carbonate.	Fibrin.	Urostealith.	Cystin.	Xanthin.	Ammonium urate.	Uric acid.

The classification of urinary concretions is based on the chemical constituents of which they are composed. They are principally those noted in the preceding table of Heller.

Before examining a calculus chemically, a thorough optical examination should be made, as this latter may give a decided clue to its composition.

Powder the calculus to a fine powder and examine it according to the preceding table.

FÆCES.

The residue which remains after complete digestion and absorption in the intestine differs, of course, according to the quantity and quality of the food. The constituents of the fæces are for the most part as follows: (1) digestible or absorbable constituents of food, which have not had time to become digested or absorbed in the digestive tract; (2) indigestible bodies, such as cellulose, keratins, and nucleins; (3) form-elements; (4) constituents of the different secretions; (5) mineral bodies; (6) products of putrefaction, such as skatol and indol; (7) micro-organisms.

In an examination of the fæces attention need be paid, clinically, to only a few points.

1. *The color.*—This is of some importance, inasmuch as the normal light- or dark-brown color may be replaced (1) by a very dark brown color (due to presence of hæmatin); (2) by a gray or slate color (due to presence of fat or absence of bile); (3) by a black (iron), yellow (rhubarb), green (mercury), due to excretion of certain substances under the influence of drugs.

2. *Presence of fats.*—This is an important clinical point, connected particularly with pancreatic disease. In this case large amounts of fat, in the form of white lumps much resembling lard, will be noticed.

3. *Total nitrogen content.*—This estimation is of the greatest importance in the study of nitrogenous equilibrium, the condition in which the nitrogen of the excretion equals that of the food taken in. The nitrogen of the food is converted partly into urea and partly into other waste products, which latter pass out as

constituents of the fæces. The total nitrogen content of the food minus the nitrogen content of the urine gives the nitrogen of the fæces. Determine this nitrogen by Kjeldahl's method (see "Urine").

4. *Presence of mucous casts.*—This is important in the diagnosis of certain forms of intestinal disease and should be looked for in doubtful cases of mucous colitis.

5. *Presence of Amoebae coli.*—These should be looked for in every case of dysentery, as their presence may aid in diagnosis of the disease.

6. *Intestinal worms.*—It is of the greatest importance to examine the fæces for these worms before a proper treatment can be instituted. Various worms are found, at times, in the fæces. Among these are (1) the various tæniæ (tape-worms), (2) *Ascaris lumbricoides,* (3) *Oxyuris vermicularis* (pin-worm), and (4) distomata. Consult special text-books for the characteristics of each, and those of their eggs.

APPENDIX.

The following reagents, contained in properly labeled stoppered bottles, are provided for each set of three students:

Sodium hydrate, 10 per cent.
Sodium carbonate, 10 per cent.
Ammonium hydrate, 25 per cent.
Hydrochloric acid (concentrated), 32 per cent.
Hydrochloric acid, 10 per cent.
Sulphuric acid (concentrated), 92 per cent.
Sulphuric acid, 10 per cent.
Nitric acid (concentrated), 68 per cent.
Acetic acid, 10 per cent.
Ammonium oxalate, 2 per cent.
Lead acetate, 2 per cent.
Copper sulphate, 2 per cent.
Ferric chloride, 2 per cent.
Potassium ferrocyanide 1 per cent.
Haines's solution.
Millon's reagent.
Magnesium sulphate (solid).
Ammonium sulphate (solid).

Besides the above, the following reagents, which are not so generally used, are placed on side shelves:

Silver nitrate, 1 per cent.
Sodium chloride, 10 per cent.
Phenol phthalein, alcoholic solution.
Ammonium molybdate, 10 per cent.
Barium chloride, 5 per cent.
Calcium chloride, 5 per cent.
Magnesium sulphate, 10 per cent.
Sodium sulpho-cyanate, 2 per cent.
Sodium nitro-prusside, 1 per cent.
Tannic acid, ½ per cent.
Phospho-tungstic acid, 2 per cent.
Bromine (saturated aqueous solution).
Ferrous sulphate, 5 per cent.
Mercuric chloride, 1 per cent.
Potassium bichromate, 2 per cent.
Iodine in potassium iodide.
(7 g. iodine, 13 g. potassium iodide in 700 c.c. water.)
Potassium iodide, 10 per cent.
Potassio-mercuric iodide.
Picric acid (aqueous), ½ per cent.
α-naphthol (alcoholic solution), 2 per cent.
Ammonium sulphide solution.
Sodium phosphate, 5 per cent.

The following apparatus is given out to each student:

1 tripod.
2 wire gauzes.
1 iron stand with 3 rings.
1 muff and clamp.
1 Bunsen burner.
1 test-tube rack.
12 test-tubes.
1 filter flask.
1 porcelain sieve.
1 glass plate.
1 graduate 50 c c.
4 fermentation tubes.
1 thermometer.
1 pair brass forceps.

2 funnels.
1 nest of beakers.
1 wash-bottle.
2 Erlenmeyer flasks.
1 pipette.
2 glass rods.
3 evaporating dishes.
2 watch-glasses.
1 bell-jar.
1 Kjeldahl flask.
1 file.
2 clay triangles.
1 test-tube brush.
50 filter papers.
1 mortar and pestle.
1 crucible and cover.
1 nickel spatula.
1 burette.
2 500-c.c. flasks.

The following apparatus is given out on temporary orders:

Spectroscope.
Thoma-Zeiss blood-counting apparatus.
Fleischel hæmoglobinometer.
Doremus's ureometer.
Esbach's albuminometer.
Einhorn's saccharimeter.
Babcock's apparatus.
Hæmatokrit.
Specific-gravity apparatus (Squibb's).
Polariscope.
Microscope.
Carbon dioxide generator.
Hydrogen sulphide generator.
Distillation apparatus.
Kjeldahl flask.
Dialyzing tubes.

Supplies beyond ordinary shelf reagents required in the various chapters of this book. Estimates based on 100 students:

Chapter I.

500 cakes of compressed yeast.
200 eggs.
80 kilos alcohol.
20 kilos ether.
10 kilos acetone.
1 kilo fusion mixture (1 part potassium nitrate and 2 parts sodium carbonate).
5 L. 10 per cent. alcoholic sodium hydrate.
2 kilos commercial glucose.
50 g. metallic sodium.
100 g. phenyl hydrazine hydrochlorate.
2 L. solution b.
2 L. solution c.
40 L. solution d.
200 sterile agar-agar tubes.
200 sterile meat-juice tubes.
200 sterile glucose-agar-agar tubes.
Cultures of *Bacillus prodigiosus, coli communis,* and *typhosis.*

Chapter II.

1 pint olive oil.
1 lb. butter.
10 kilos alcohol.
1 lb. mutton tallow.
1 L. Barfoed's reagent.
100 g. starch.
100 g. dextrin.
1 gallon blood serum.

5 kilos ether.
5 kilos chloroform.
1 L. soap solution.
1 L. 10 per cent. alcoholic sodium hydrate.
100 g. glucose.
100 g. lævulose.
100 g. cane sugar.
100 g. lactose.
100 g. maltose.
20 lbs. lean meat.
2 kilos Witte's peptone.
½ gallon of milk.
2 kilos commercial peptone.
2 kilos barium hydrate.
4 kilos blood fibrin.
2 kilos animal charcoal.
150 g. phospho-tungstic acid.
10 kilos horn.
100 g. egg albumin.

Chapter III.

200 g. starch.
1 L. natural gastric juice.
25 L. artificial gastric juice.
20 lbs. fibrin.
1 lb. butter.
1 gallon milk.
5 L. 1 per cent. cane sugar solution.
4 L. neutral pepsin solution.
1 L. *n*-10 hydrochloric, sulphuric, nitric, acetic, oxalic, phosphoric, and lactic acids.
100 g. papain.
50 c.c. Töpfer's reagent.
50 c.c. Gunzburg's reagent.
50 c.c. Boas's reagent.
50 c.c. tropæolin 00 solution.
50 c.c. congo-red solution.
4 L. *n*-10 sodium hydrate.
50 c.c. aqueous solution of alizarin.
20 g. potassium iodide.
10 g. iodoform.
100 g. salol.
25 L. artificial pancreatic juice.
100 c.c. olive oil.
10 L. ox's bile.
100 c.c. dog's bile.
5 L. alcohol.
5 L. ether.
10 lbs. pancreas.
5 lbs. intestine.

Chapter IV.

½ gallon defibrinated ox blood.
2 gallons oxalated horse's blood.
100 c.c. tincture of guaiacum.
100 c.c. ozonized turpentine.
200 c.c. hydrogen peroxide.
20 kilos ox liver.
5 kilos ox kidney.
60 kilos lean meat.
5 kilos Armour's or Liebig's extract of beef.
200 sheep's brains.
5 kilos pancreas.
½ kilo pepsin.
10 kilos alcohol.
10 kilos ether.
10 kilos acetone.

Chapter V.

1 L. Stutzer's reagent.
1 gallon milk.

CHAPTER VI.

2 L. standard silver nitrate solution. (29.075 g. in 1 L.)

2 L. standard potassium sulphocyanate solution (8.3 g. in 1 L.).

1 kilo solid barium carbonate.

2 L. uranium nitrate solution (44.78 g. in 1 L.).

6 L. sodium hypobromite solution.

2 L. magnesia mixture.

3 kilos ammonium chloride.

100 c.c. alcoholic zinc chloride solution.

8 L. *n*-10 sodium hydrate.

1 lb. Squibb's solution of chlorinated soda.

100 c.c. trichloracetic acid.

8 L Fehling's quantitative solution.

2 L. saturated sodium chloride solution.

100 g. phenyl hydrazine hydrochlorate.

100 g. sodium acetate.

50 cakes of yeast.

100 g. bismuth subnitrate.

2 L. lime-water.

The following tables will be of use in converting the English system of weights and measures into the metric system, and *vice versa:*

WEIGHTS.

ENGLISH INTO METRIC.

1 grain = .0648 grams.
1 ounce = 437.5 grains
= 28.3595 grams.
1 pound = 7000 grains
= 453.5925 grams.

METRIC INTO ENGLISH.

1 milligram = .015432 grains
1 centigram = .154323 "
1 decigram = 1.543235 "
1 gram = 15.43235 "
1 decagram = 154.3235 "
1 hectogram = 1543.235 "
1 kilogram = 15432.35 "

CAPACITY.

METRIC INTO ENGLISH.

1 minim = .059 cubic centimeters
1 fluid drachm = 3.549 " "
1 fluid ounce = 28.398 " "
1 pint = 567.936 " "
1 gallon = 4548.37 " "

ENGLISH INTO METRIC.

1 cubic centimeter = 16.931 minims.
1 Liter (1000 c.c.) = 35.2154 fluid ounces
= 1 pint 15 ounces, 2 drachms 11 minims.

PLATES I-XXI.

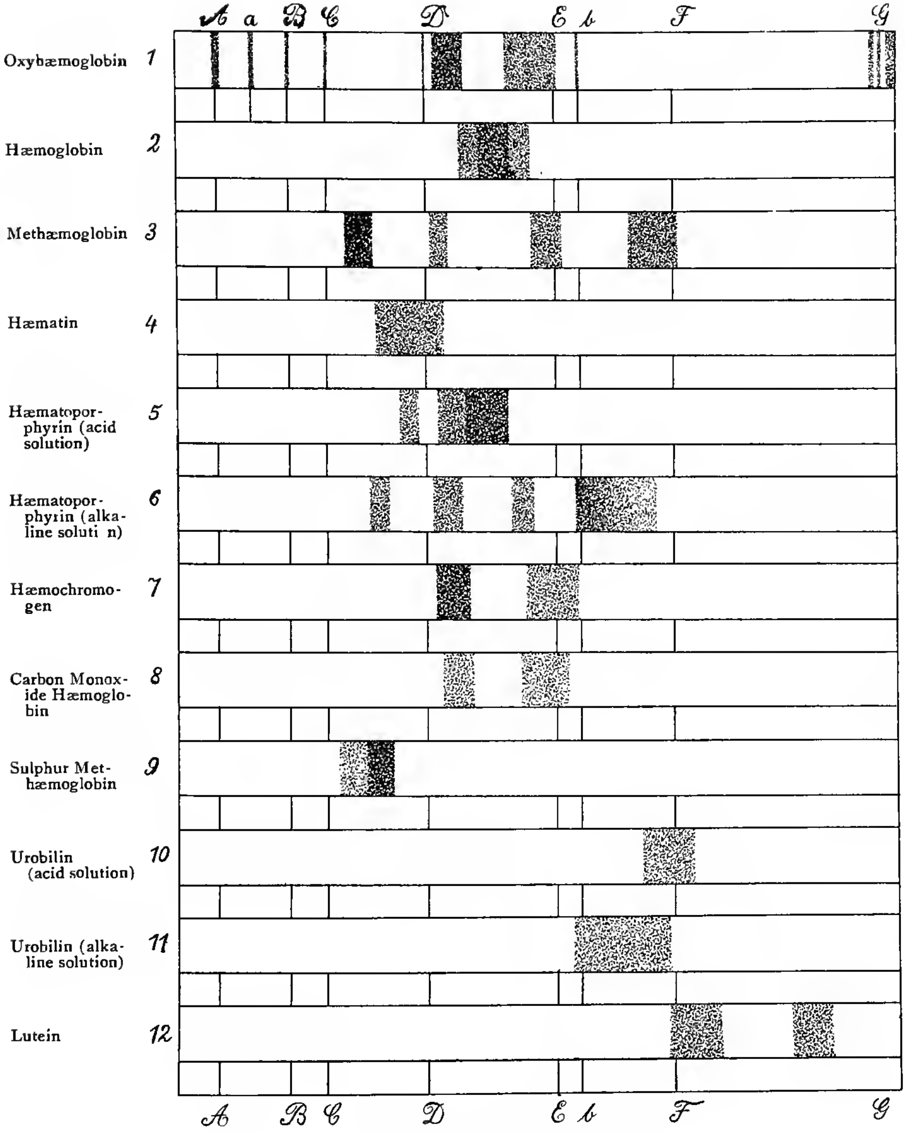

A a B C D E b F G
Oxyhæmoglobin 1
Hæmoglobin 2
Methæmoglobin 3
Hæmatin 4
Hæmatopor-phyrin (acid solution) 5
Hæmatopor-phyrin (alkaline solution) 6
Hæmochromogen 7
Carbon Monoxide Hæmoglobin 8
Sulphur Methæmoglobin 9
Urobilin (acid solution) 10
Urobilin (alkaline solution) 11
Lutein 12
A B C D E b F G

PLATE I. ABSORPTION SPECTRA.

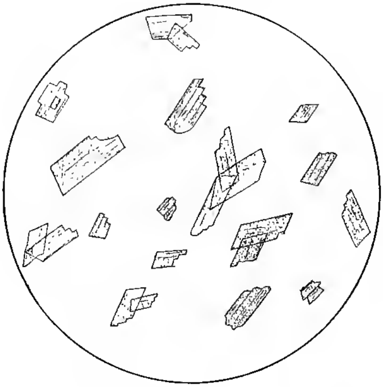


PLATE II. CHOLESTERIN CRYSTALS.

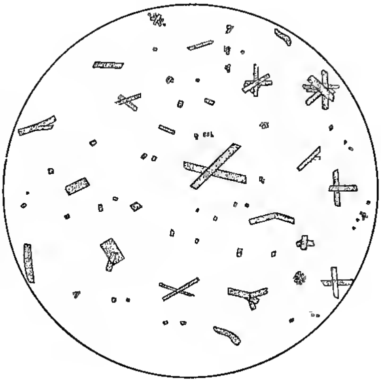


PLATE III. HÆMIN CRYSTALS.

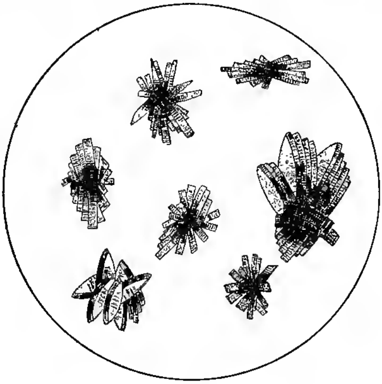


PLATE IV. URIC ACID.

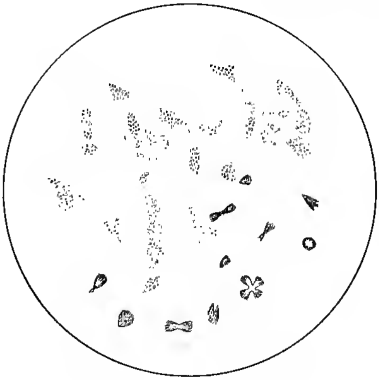


PLATE V. SODIUM AND POTASSIUM URATES.

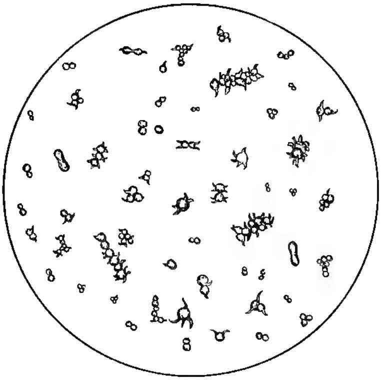


PLATE VI. AMMONIUM URATES.

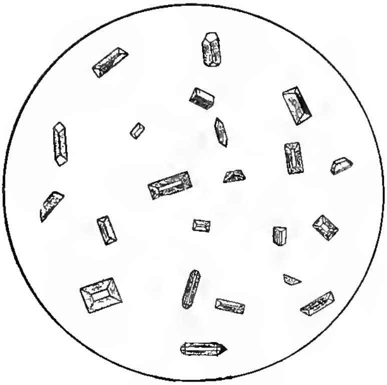


PLATE VII.
MAGNESIUM AMMONIUM PHOSPHATE.

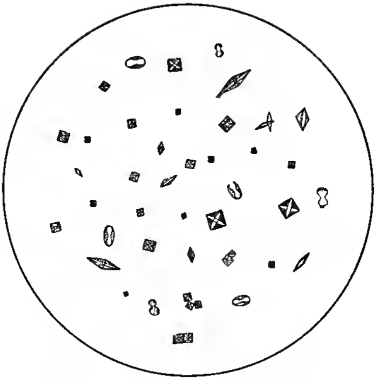


PLATE VIII. CALCIUM OXALATE.

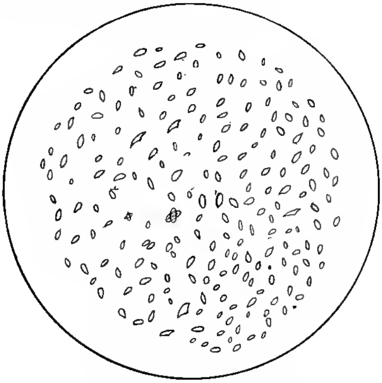


PLATE IX. XANTHIN.

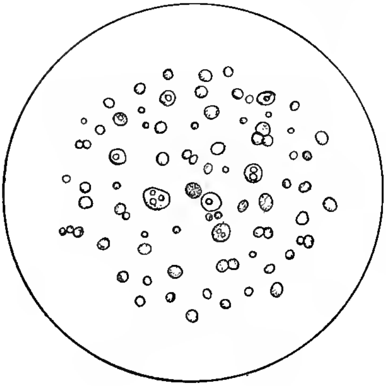


PLATE X. LEUCIN.

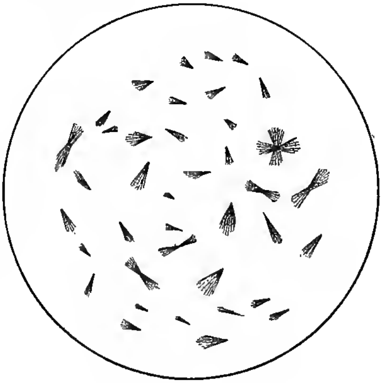


PLATE XI. TYROSIN.

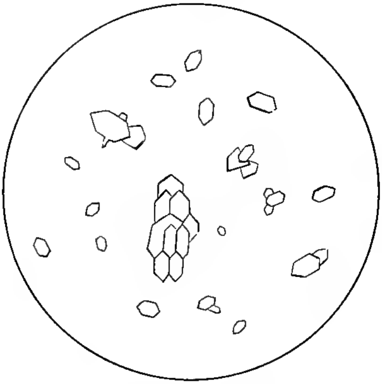


PLATE XII. CYSTIN.

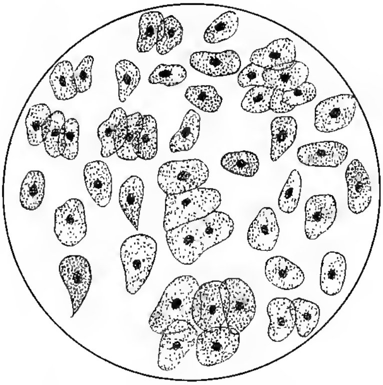


PLATE XIII. EPITHELIAL CELLS.

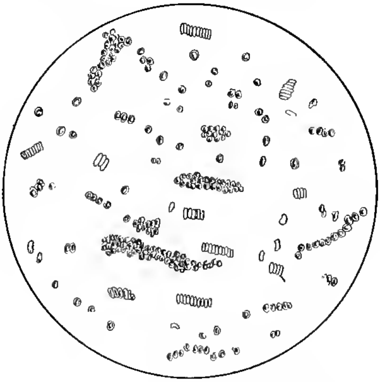


PLATE XIV. RED BLOOD-CORPUSCLES.

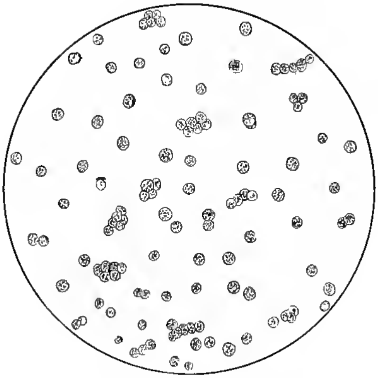


PLATE XV. LEUCOCYTES (PUS).

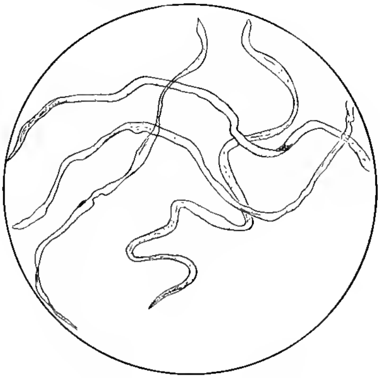


PLATE XVI. CYLINDROIDS.

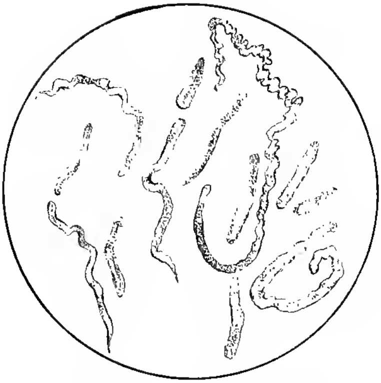


PLATE XVII. HYALINE CASTS.

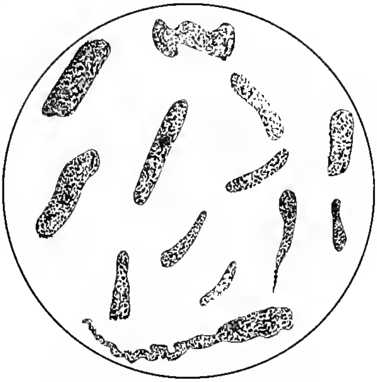


PLATE XVIII. GRANULAR CASTS.

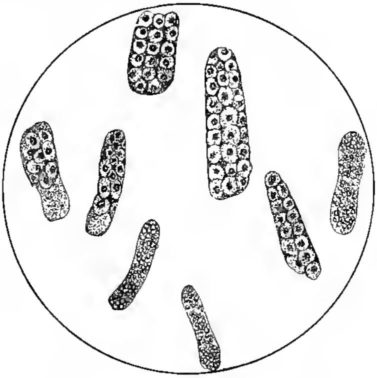


PLATE XIX. EPITHELIAL CASTS.

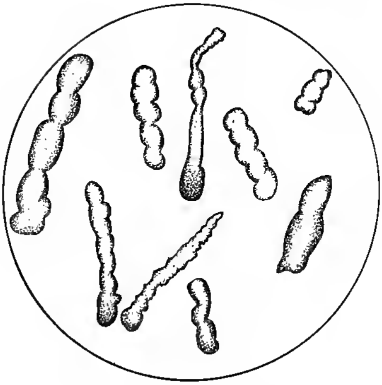


PLATE XX. WAXY CASTS.

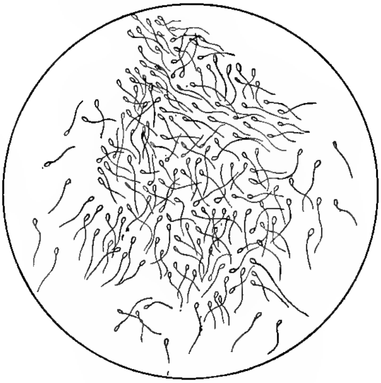


PLATE XXI. SPERMATOZOA.

INDEX.

Absorption from stomach, 37.
Acetic acid, 34.
Aceto-acetic acid, 84.
Acetone, 85.
Acid albumin, 18, 22.
Acidity of urine, 76.
Acrolein, 2, 13.
Acroodextrin, 29.
Adamkiewicz's reaction, 5.
Albuminoids, 16.
Albumins, 16.
Albuminuria, 77.
Albumoses, 16-21.
Alizarin, 36.
Alkali albumin, 16.
Ammonia in urine, 66.
Ammonium magnesium phosphate, 90.
Ammonium urate, 90.
Amoebae coli, 94.
Amylodextrin, 29.
Amyloid, 16.
Amylopsin, 38.
Arginin, 23.
Artificial gastric juice, 30.
Aspartic acid, 22.
Bacillus coli communis, 11.
Bacillus prodigiosus, 10.
Bacillus typhosus, 11.
Barfoed's reagent, 14.
Benzoyl-cystin, 25.
Bile, 39.
Bile acids, 39.
Bile pigments, 39.
Biliary calculi, 40.
Bilirubin, 39.
Biliverdin, 39.
Bismuth subnitrate test, 6, 82.
Biuret test, 5, 21, 79.
Blood, 42, 87.
Blood corpuscles, 45, 91.
Blood serum, 17, 45.
Boas's test, 34.
Bone, 18, 48.
Borax in milk, 60.
Böttger's test, 82.
Brain, 54.
Butyric acid, 34.
Calcium, 7.
Calcium oxalate, 88.
Calcium phosphate, 90.
Cane sugar, 14, 84.
Caproic acid, 12.
Caprylic acid, 12.
Carbohydrates, 6, 14, 59, 81.
Carbon-monoxide hæmoglobin, 43.
Casein, 16, 19.
Casts, 91.
Cephalin, 1, 12, 55.
Cerebrin, 55.
Cetyl alcohol, 12.
Chlorides, 28, 63.
Chlorophyl, 44.
Cholesterin, 3, 12, 55.
Cholin, 2, 12.
Chondroitin sulphuric acid, 16.
Chromo-proteids, 16.
Clotting of blood, 45.
Coagulation test for proteids, 4, 77, 81.
Collagen, 16, 18.
Combined acids, 36.
Compound proteids, 16.
Congo-red, 34.
Corn starch, 6.
Cystein mercuric chloride, 25.
Cystin, 24.
Cytoglobulin, 16.
Deutero-albumose, 16, 21, 33, 38.
Dextrin, 6, 15.
Dextrose, 6, 14, 28, 81.
Diacetic acid, 84.
Di-amido acids, 23.
Digestive power of acids, 32.
Digestion, 27.
Dimethyl-amido-azo-benzol, 33, 36.
Disaccharides, 14.
Edestin, 18.
Egg albumin, 4, 16.
Elastin, 16.
Embden's method for cystin, 24.
Emulsification, 39.
Entero-kinase, 41.
Epithelial cells, 91.
Erepsin, 41.
Erythrodextrin, 29.
Esbach's method, 81.
Ewald's test, 37.
Fæces, 93.
Fats, 1-12.
Fatty acids, 13.
Fehling's test, 6, 14, 82, 83.

Fermentation, 7, 8.
Fermentation test, 82.
Ferrocyanide test, 5, 78.
Fibrin, 16, 45.
Fibrinogen, 16, 45.
Fibroin, 16.
Fleischer's test, 37.
Folin's method for ammonia, 66.
Foods, 12.
Formaldehyde in milk, 61.
Fructose, 14, 84.
Gastric digestion, 30.
Gastric juice, 30.
Gerhardt's test for diacetic acid, 84.
Gelatin, 16, 18.
Globin, 16.
Globulins, 16, 17.
Glucose, 6, 14, 28, 81.
Glutaminic acid, 22.
Glycerin, 2, 13.
Glycogen, 15, 46.
Glycoproteids, 16, 19.
Glycosuria, 81.
Gmelin's test for bile pigments, 39, 86.
Guaiacum test, 42, 87.
Gunzburg's test, 34.
Hæmin crystals, 43.
Hæmochromogen, 43.
Hæmatoporphyrin, 44.
Hæmoglobin, 16, 43.
Haines's test, 6, 14, 82.
Haines's coefficient, 66.
Haser's coefficient, 66.
Hay's test for bile acids, 86.
Heat test for proteids, 77.
Heller's test for proteids, 4, 77, 81.
Hetero-albumose, 21, 33, 38.
Hexon bases, 23.
Histidin, 23.
Histon, 16.
Hoffman's test for tyrosin, 24.
Hopkin's method for uric acid, 71.
Horn, 24.
Humin, 22.
Huppert's test for bile pigments, 40, 86.
Hydrochloric acid, 31, 33, 36.
Hypoxanthin, 52, 73.
Indican, 75.
Inorganic salts, 7, 58, 63.
Intestinal juice, 41.
Intestinal worms, 94.
Inversion, 7, 14.
Invertin, 7.
Jaffe's reaction
  for indican, 76.
  for kreatinin, 51, 74.
  for urobilin, 75.
Kelling's test for lactic acid, 35.
Keratin, 16.
Kidney, 47.
Kjeldahl's method for nitrogen, 67.
Kossel's test for hypoxanthin, 73.
Kreatin, 49.
Kreatinin, 49, 73.
Kreatinin zinc chloride, 49, 73.
Külz's test for β-oxy-butyric acid, 84.
Lact-albumin, 16, 58.
Lactic acid, 34.
Lactose, 15, 59, 84.
Lævulose, 14, 84.
Lecithin, 1-55.
Lecitho-proteids, 16.
Legal's test for acetone, 85.
Le Noble's test for acetone, 85.
Leucin, 23, 25.
Leucin lead oxide, 24.
Leucocytes, 91.
Lieben's test for acetone, 85.
Liebermann's reaction for proteids, 6.
Lipase, 38.
Liver, 46.
Lysin, 23.
Magnesium, 28.
Magnesium-ammonium phosphate, 90.
Magnesium phosphate, 90.
Maltose, 15, 28.
Melanoidinic acid, 22.
Methæmoglobin, 43.
Milk, 57.
Millon's reagent, 5.
Mono-amino acids, 23.
Monosaccharides, 14.
Mörner's test for tyrosin, 24.
Motor power of stomach, 37.
Mucin, 16, 19, 28.
Mucous casts, 94.
Murexid test, 70, 73.
Muscle, 49.
Mutton tallow, 13.
Myo-albumin, 51.
Myoglobulin, 51.
Myosin, 16, 51.
Myosinogen, 16, 51.
∝ naphthol test, 7.
Nitrogen in urine, 67.
Nucleic acids, 3.
Nucleo-albumins, 16, 19.
Nucleo-proteids, 16.
Obermayer's test for indican, 76.
Oleic acid, 12.
Olive oil, 13.
Organic acids, 12.
Ossein, 19.
β-oxy-butyric acid, 84.

Oxyhæmoglobin, 43, 46.
Palmitic acid, 12.
Pancreas, 55.
Pancreatic juice, 37.
Pancreatic digestion, 37.
Papain, 32.
Paralactic acid, 54.
Paramyosinogen, 51.
Paranucleic acid, 16.
Pasteur's experiment, 10.
Pentose, 84.
Pepsin, 31.
Peptone, 21, 23, 33.
Pettenkoffer's reaction for bile acids, 39, 86.
Phenyl-hydrazine test for carbohydrates, 6.
Phosphates, 64.
Phosphoric acid, 2, 64.
Phosphorized albumins, 16, 19.
Picric acid test for proteids, 5, 79.
Piria's test for tyrosin, 24.
Plasma, 42.
Polarization, 15.
Potassium urate, 88.
Primary albumoses, 20.
Protalbumose, 20, 33, 38.
Protamine, 5.
Proteids, 4-16, 58, 77.
Ptyalin, 29.
Purdy's test for proteids, 78.
Rennin, 30, 31.
Reticulin, 16.
Reynold's test for acetone, 85.
Rosenbach's test for bile pigments, 86.
Salicylic acid in milk, 60.
Saliva, 27.
Salivary digestion, 27.
Salkowski's method
  for sulphates, 65.
  for xanthin, 73.
Saponification, 13.
Sarco-lactic acid, 54.
Scherer's test for leucin, 23.
Schiff's reaction, 71.
Secondary albumoses, 16, 21, 33, 38.
Seliwanoff's test for lævulose, 14.
Serum-albumin, 16, 17, 45.
Serum-globulin, 16, 17, 45.
Simple proteids, 16.
Soap, 2.
Sodium carbonate, 66.
Sodium urate, 88.
Soluble starch, 29.
Specific gravity
  of milk, 57.
  of urine, 63, 66.
Spectra, 43.
Spitzer's oxydase, 47.
Splitting products of proteids, 22.
Starch, 15.
Steapsin, 38.
Stearic acid, 12.
Stomach, 56.
Struve's test for blood, 87.
Stutzer's reagent, 59.
Sulphates, 65.
Sulpho-cyanate of potassium, 28.
Sulphur, 25.
Syntonin, 18, 22.
Teichmann's crystals, 43.
Töpfer's test for hydrochloric acid, 33.
Total solids
  milk, 57.
  urine, 66.
Trichloracetic acid test for proteids, 78.
Trommer's test for sugar, 81.
Tropæolin oo test for hydrochloric acid, 34.
Trypsin, 37, 55.
Tryptophan, 23.
Tyrosin, 24, 38.
Uffelmann's test for lactic acid, 35.
Ultzmann's test for bile pigments, 86.
Urea, 67.
Urea nitrate, 68.
Uric acid, 70.
Urinary calculi, 92.
Urinary sediments, 87.
Urine, 62.
Urobilin, 75.
Volhard's method for chlorides, 63.
Weidel's reaction for xanthin, 54-72.
Weyl's reaction for kreatinin, 50, 74.
Witte's peptone, 20.
Xanthin bases, 52, 72.
Xantho-proteic reaction, 5.
Zinc sarco-lactate, 54.